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PEDOGENETICAL STUDIES
OF
SOIL ORGANIC MATTER

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by

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Chapter I

INTRODUCTION

The "soil" has been defined variously by a number of scientists since the dawning of the modern science. They have refered, without exception, to the organic matter existing in the soil as a principal constitutional element, that is to say, they have thought that presence of the organic matter is one of the discriminative features of a "soil" from a "parent material".

According to the modern concept of a soil, it is defined as a historical natural body formed under the influence of the following factors; living organism, climate, parent material, topography and time. There might predominate the effects of the climatic factor in one soil, and those of the parent material in another, but in all the soils the biological factor is most important than the other, for in any case where there is no living organism, there does not exist a "soil" in the strict sense.

The formation of the soil organic matter depends on activities of the living organisms, which are in turn regulated by the other factors of soil formation, so all the processes working in the soil are reflected in the properties of the soil organic matter. Therefore, studies of the soil organic matter often lead to a right understanding of the soil itself. Onecan easily find out a typical example in the history of soil science.

Towards the close of the 19th century, a famous Russian investigator V.V. Dokuchaev carried out an extensive study on the Russian chernozems and found a geographical regularity of distribution of the humus in the soils, that is, a fact that the humus content of the soils diminishes gradually from south to north corresponding to the transition of the climatic zones. In this way Dokuchaev gained a clue for recognizing the principle of "zonality" of the soil, which is one of the basic ideas of the modern soil genesis.

Thus, studies on quantity and/or quality of the soil organic matter afford important informations relative to genetical understanding of the soil. It is largely for this reason that attention of many soil scientists has long been focused on this soil constituent.

Nevertheless, there still remain many undissolved problems, especially in dynamic aspects of the organic matter-soil relations. Many workers dealt with the organic matter for its own sake and payed no close attention to the soil in which the organic matter is contained, or at best they could draw an "inference" on the relations between them relying on the results of their static studies. Although the author has been eager to know: "What are the modes of association of the organic matter with the mineral constituent in the soil in situ ?" or "How proceeds the process of the humus formation under the given soil conditions ?". These questions have very rarely been answered.

In order to answer these questions by himself the author intended to carry out the experiments described below. Though the author's intention was not always perfectly fulfilled, some results obtained would contribute to the development of the knowledge on the soil organic matter.

First of all, the author will give a brief review on the modern concepts of the soil organic matter, especially of the nature and properties of the humus and of the behavior of the soil organic matter as complexing agents (Chapter 2). These subjects have been attracting a growing interest of the workers.

Then the author will present his own experiments on the association of the humus with mineral constituents in the main soil groups in Japan. A bonding mechanism involved in the association will also be presented (Chapter 3 and 4).

Considerations on these subjects induced an argument on the structure of the humic substances. The author will present his data of the neutralization capacity of some humus preparations and discuss the structural problem (Chapter 5).

Then, turning into the problem of the humus formation in the volcanic ash soils, which distribute widely in this country, the author will deal with an adsorption of polyphenols on a volcanic ash material and the initial stage of their alteration (Chapter 6).

The author carried out another experiment, in which the effects of polyphenols and a polyuronids on the soil formation were examined using soil columns (Chapter 7).

Before entering into the main discourse, the author will give an

explanation of the terminology used hereafter.

As described above, the soil organic matter is derived from the living organisms residing both in and on the soil, and therefore, includes a wide variety of organic compounds. Some authors regard this variety of organic materials collectively as humus [84].

However, here the author will define the humus (or humic substances) as follows; the humus is a collective designation of the inherent organic material in the soil. It has a complicated structure, a fairly high molecular weight and an acidic nature. Blackish color, colloidal behavior, cation exchange ability, and high resistance to microbial decomposition are also its distinctive properties.

The humic substances are conventionally divided into two fractions, humic acids and fulvic acids, as in the routine fractionation. However, the customary definition of fulvic acids has been rather uncertain, because the fulvic fraction obtained with the usual procedure contains non-humic substances besides humic [30] (the definition in a broader sense). However, fulvic acids have to originally be one fraction of the "humic substances" and in the following discourse the author will use the term "fulvic acids" in this strict sense unless otherwise mentioned. So-called humine is thought to be no other than humic acids which are strongly attached to the soil mineral fraction——clays and sesquioxides.

The remaining part of the soil organic matter after eliminating the humus is non-humic substances. Though the chemical nature of the substances contained in this fraction can not be fully grasped, there might exist many compounds coming into almost all the categories of the modern organic chemistry.

In conclusion, the soil organic matter consists of two fractions, humic and non-humic fraction; then the humic fraction consists of also two parts, humic acids and fulvic acids.

Chapter 2

A REVIEW ON THE MODERN CONCEPTS OF THE SOIL ORGANIC MATTER

2.1. The nature and properties of the soil humus

As defined in the preceding chapter the most essential requisite of humus is that it consists of a group of peculiar organic compounds in a soil. However, the meaning of "the peculiarity" would depend on the standard of one's knowledge, that is to say, a peculiarity of today might be a commonness of tomorrow owing to the advances in the knowledge. Taking such a consideration into account the author will deal with nature and properties of the soil humus relying on the present understandings.

Plant materials which are known to be the most important source of the soil humus have been studied considerably deeply and the nature of these materials has become fairly clear today. Even lignin, which represents the most complicated plant material, has been known to be condensation products of conipheryl alcohol.

Humus is a product derived from those plant materials through chemical and microbiological processes in a soil and shows a high stability against microbial decomposition unlike the original plant materials. It seems that humus is a meta-stable form in which organic carbon is accumulated on the earth surface.

Plant lignin is considerably resistant to a microbial attack and, therefore, it was once thought to be the direct source of humus, for example, in Waksman's ligno-protein theory [99] and Mattson's lignin-ammonia concept [70, 71]. However, the stability of humus in the soil is far greater than that of lignin and this fact can be taken as one of the evidences which indicate that humus is not a mere denaturated or a derived product of the original plant materials, but is a product synthesized through special processes characteristic to the soil.

Such an idea of the nature of humus is supported by many investigators working today all over the world. Scheffer [84] and Flaig [29] in Germany, Bremner [20] in England, Swaby [91] in Australia, Kononova [50, 51] in Russia and Kumada [60] in Japan, all these investigators agree with each other in denying an exclusive significance of lignin

as the source of humus and in regarding humus as a synthesized product peculiar to a soil. They are also coincident with each other in deeming polyphenols as one of the most essential constituents of this synthesized soil humus. These polyphenols and quinoids are supplied to a soil not only as a result of decomposition of aromatic plant materials such as lignin and tannin but also in the form of respiratory catalysts of raw plant tissue as well as products of microbial metabolisms taking place in the soil.

If the last-mentioned process is taken into consideration, it is readily noticeable that all the plant materials including the most easily decomposable ones are able to contribute to the humus formation through microbial activities. In fact, Kononova and Aleksandrova [52] indicated that humus-like substances having aromatic compounds in their composition were obtained from a liquid culture of a mould containing nothing aromatic but sugars as a carbon source.

The presence of aromatic compounds in humus as a fundamental constituent has been known since the time of Hoppe-Seyler. Many investigators identified a variety of aromatic compounds in humic substances with the aid of alkaline fusion methods or with various oxidation techniques. Recently development of IR-spectrophotometry has made it possible to prove the presence of aromatic constituents in humus with both ease and high reliability [61, 102].

Among those aromatic compounds polyphenols and quinoids are the most reactive and are easily oxidized to give oxidative polymerization products. The importance of the polyphenolic compounds as an essential constituent of humic substances is derived from the considerations mentioned here.

Another characteristic found in the constitution of humus is that it contains more or less nitrogenous components. A part of the nitrogen (normally below 50 %) is easily hydrolyzed to give amino acids and seems to be proteinaceous compounds, while another greater part (normally above 50 %) is not released by hydrolysis and is thought to be in a heterocyclic ring system. It is a general understanding that most of these nitrogenous components originate in microbial protoplasma. However, it is not yet certain that proteins and peptides undergo condensation with polyphenols whether in their original forms or, after being autolytically decomposed, in the form

of amino acids, which, according to Swaby [91, 92], get into cross linking with polyphenols through their amino groups.

In any case it is now generally understood that these two constituents——polyphenols and nitrogenous compounds——are the most essential ones of humic substances.

However, the mechanism by which these constituents bind each other, as well as the structure of the product are scarcely known. Condensation between polyphenols and nitrogenous compounds might occur by either a biochemical reaction catalysed by microbial oxidases or a purely chemical one, and it seems likely that both are the cases.

Humic substances thus obtained are quite diverse in their many properties, on the one hand, but, on the other, they are regarded as one characteristic group constituted on one and the same principle. Both the diversity and the common feature will be noticed in the following description.

The elementary composition of the humic substances has a wide diversity, for example, the carbon content ranges from about 45 % in fulvic acids to about 60 % in humic acids. According to Russian investigators [51] changes in the elementary composition of humic acids follow regularly to the soil zonality, for example, the carbon content gradually increases from that of podzol humic acids in the taiga zone to that of chernozem humic acids in the steppe and at the same time ratio of oxygen content to hydrogen becomes greater from the taiga to the steppe, indicating progress of oxidation as well as dehydration process.

Such a relation between the nature of humic acids and the soil type is also recognized in their optical property and in their colloid-chemical behavior. Along with the transition of the zonal soil types from podzol to chernozem the optical density of the humic acid solution becomes gradually greater and, at the same time, sensibility to the electrolytes increases, which indicates lowering of dispersibility. However, in their absorption spectrum a common feature is noticed, that is to say, curves of the absorption spectra monotonously descend from a height at the UV-region towards the longer wavelength region and show no clear absorption maxima or minima. Moreover, IR-spectum and X-ray diffraction analysis can afford additional evi-

dences for that humic substances are constituted on the same constituting principle.

In order to explain both the diversity in the properties and the common constituting principle of the humic substances, Russian investigators adopted a schematic model, as shown in Figure 2.1., which was originally proposed by Kasatochkin for the carbonized materials [43, 50]. All the humic substances have an aromatic nucleus and aliphatic side chains in their molecule, and in the more humified substances like chernozem humic acids the aromatic nucleus is relatively predominating and in the less humified ones like fulvic acids the peripheral chains containing many hydrophilic groups become dominant. The reciprocal relation between the nucleus and the side chains lucidly explains the diversity of the various properties mentioned above.

In Japan Kumada [56 - 59] carried out an extensive study on the humic acids of main soil groups in Japan——humus volcanic ash soils, dry paddy soils, wet paddy soils and peaty soils. He found that the absorption spectrum of a humic acid preparate was its most essential attribute and that the optical density increased in the following order;

peaty soils < wet paddy soils < dry paddy soils < humus volcanic ash soils

At the same time the slope of the curves represented by a $\Delta \log K$ value ($\Delta \log K = \log K_{400\ m\mu} - \log K_{600\ m\mu}$) was arranged just in a

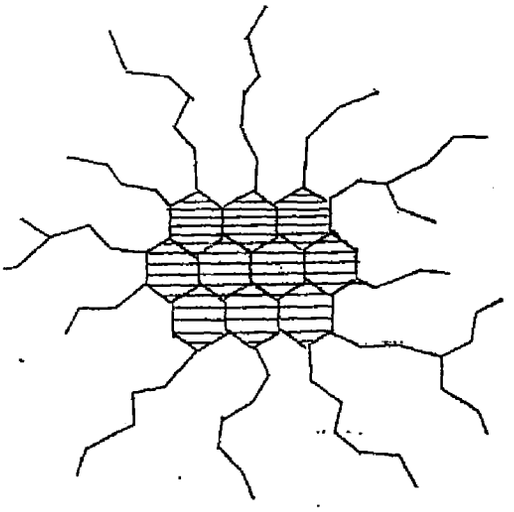


Fig. 2.1. A model of the structure of carbonized materials including humic substances (Kasatochkin, V.I.)

reverse order. The Δ log K value corresponds to Springer's [89] color quotient (FQ) and represents a humification degree, that is to say, the higher the value is, the less humified is the humic acid preparate.

The soil series thus arranged according to the Δlog K value shows a good correspondence to the regularity of the variation found in several properties such as elementary composition, exchange capacity, proportion of the hydrolyzable nitrogen, sensibility to electrolytes, etc., of humic acids obtained from the soils in the series.

The results obtained by Kumada are, as a whole, thus concordant with those by Kononova. However, they are different from each other in the following point: Kumada [60] postulated two types of humification process; the one is related to the more humified humic acids found in the humus volcanic ash soil. They are formed from polymerization products of phenolic compounds, which originated in microbial metabolic substances. The other concerns with the less humified humic acids often found in paddy and peaty soils. These humic acids are derived principally from lignin, which degenerates in the soil without undergoing marked change in its skeletal structure and gradually humifies taking microbial products in its own body. On the contrary, Kononova [51] postulated that all the humic substances are formed according to one and the same constituting principle, and the forming process is a synthetic one taking place in the soil.

Then, what relations has the nature of humic substances with the role played by them in the process of soil formation ? Russian investigators [51] gave an explanation as follows; under an excessively humid condition like that found in northern taiga zone condensation between polyphenols and nitrogenous compounds does not go farther owing to the difficulty in removing the by-product of the reaction——water, and thus the products seem to have an undeveloped aromatic nucleus and, therefore, seem to be high in dispersibility. Such humic substances would be found in podzolic soils and they could be very active as a soil former. On the contrary in the steppe where dry and wet conditions appear alternately, condensation reaction will proceed farther and produce humic substances low in dispersibility. These circumstances are seen in chernozemic soils. And chernozem humic acids may be inactive in soil forming processes.

However, in the soil structure formation just the reverse relations exist, that is, podzol humic acids are not effective owing to their high dispersibility, while chernozem humic acids are a very good structure forming agent. This explanation seems to be coincident with the field observations.

With respect to fulvic acids, there are a few researches carried out by Kononova [50] and Kobo and Tatsukawa [49]. From the results obtained from various experiments including elementary analysis, optical measurement and X-ray diffraction method, they regard fulvic acids as an analogue of the least humified humic acids, and seem to suppose that humification proceeds linearly from fulvic to humic acids.

As to humine there are still less studies, but Russian investigators [51] suggested that humine is no other than humic acids which are firmly bound to soil mineral constituents, and thus lose solubility in an alkaline solution.

Taking these explanations into consideration fulvic acids, humic acids, and humine seem to belong to one and the same polymerization homologue and seem to be conventionally divided into three parts according to their behavior towards a few solvents.

Association of humic substances with mineral constituents will be dealt with in Chapters 3 and 4, and the structural problems of humic substances in Chapter 5.

An increasing knowledge about soil humus is now accumulating and it is very difficult to command the perfect view on it. In this paragraph a concise review on the nature and properties of humus has been given.

2.2. The behavior of the soil organic matter as a complex-forming agent

Many functions displayed by the soil organic matter are thought to originate in an important ability possessed by them, that is, a complex-forming ability.

The complex-formation by the soil organic matter is a matter of much interest in many fields of soil science; e.g., extraction of humic substances from soils, cation exchange capacity exhibited by humus, the role played by organic matter in soil-forming processes and

structure formation, availability of mineral nutrients, especially of microelements in organic soils, etc. Scheffer et al. [78] reviewed the papers dealing with chelates and chelation in soil science, and Bremner [21] spared a relatively wide space for discussing the complex-formation in his review on the soil organic matter problems. Recently the authors [47] have also prepared a review on the same theme.

Here the behavior of the soil organic matter as a complex-forming agent will be concisely described with special reference to the subjects to be treated in Chapters 4 and 7.

i) Complex-formation by humic substances in a soil

In 1946 Bremner et al. [18] suggested a theory that, in soils, polyvalent metals are combined with organic matter as metal-organic matter complexes, and that these complexes are insoluble in solvents that do not themselves form complexes with metals. The principle of one of the most prevailing methods for humus extraction, the neutral sodium pyrophosphate extraction proposed by Bremner and Lees [19], is a corollary of the theory.

Broadbent and Ott [22] used the measurement of absorption spectra (Job's continuous variation method) [66] for ascertaining complex formation between soluble humus (fulvic acids) and Cu^{+2}. However, they could not obtain clear results on account of uncertain compositions of the fulvic acids (in a broader sense).

Broadbent [23] applied a technique of the ion exchange chromatography (using humus preparations as the ion exchanger) to his studies on metal-organic matter complexes and found that the elution curves of Cu^{+2} by hydrochloric acid solutions were different from those of Ca^{+2} in their shape. He concluded that in the formation of the complexes between the humus and Cu^{+2} certain functional groups of the humus which did not participate in the case of Ca^{+2}, should play no small part.

Beckwith [4] studied complex formation between humus and some divalent cations (Cu^{+2}, Mn^{+2}, Co^{+2}, Ni^{+2}) using pH effect [66]. All metal-organic matter complexes may be considered to be formed by the displacement of one or more usually weak acidic protons of the complex-forming agent by a metal ion, and, therefore, complex-formation is always accompanied by pH decrease. The degree of this pH decrease corresponds to the stability of the complex formed. Beckwith measured the

pH decrease caused by the addition of those cations to the suspensions of acid-washed organic soils and titrated the same samples with a sodium hydroxide solution to obtain pH-titration curves. The order of the magnitude of the pH decrease was $Cu^{+2} > Ni^{+2} > Co^{+2} > Mn^{+2}$ and the same order was noticed throughout the whole range of the titration curves. The results obtained by Beckwith were fully concurrent with those of Irving and Williams [40], who studied the stability of various chelates of these divalent cations. Beckwith suggested that Fe^{+3} and Al^{+3} might have a possibility to form stable complexes with soil organic matter, but did not present any experimental proof.

Recently Martin and Reeve [68, 69] have conducted a series of experiments on soils of some podzol illuvial horizons. They extracted B-horizon soils of the podzols with an aqueous acetone solution of acetylacetone, one of the chelating agents (Martin and Reeve [67, 68]), and using the humus solutions thus prepared, obtained pH-titration curves by a technique similar to that used by Beckwith. In their studies complex formation between humus and Al^{+3}, Fe^{+3} and certain divalent cations was not confirmed.

Himes and Barber [38] used radio active zinc in their study and tried to calculate stability constants of zinc-soil organic matter complexes by two method. The stability constant obtained by them was in the range from 5.0 to 6.0.

ii) Complex-formation in weathering and soil forming processes

Mandl et al. [65] studied many organic and inorganic substances occuring in nature with respect to their ability to dissolve difficultly soluble materials by complex-formation, and suggested that this process should be very important in a soil.

Scheffer et al. [79] proved that a neutral humic acid solution dissolved a crystalline strengite ($FePO_4 \cdot H_2O$), which is very difficultly soluble in water, and thought that this should be entirely due to complex-formation, especially chelation. According to Scheffer et al. the chelating ability of the humus solution was fairly high and 1 mg C/ml solution of brown humic acids was somewhat higher in its chelating ability than M/100 EDTA solution (1.2 mg C/ml).

Besides two investigations above mentioned there are many other examples which indicate the importance of complex-forming ability of the soil organic matter in the process of weathering. However, the

importance in the soil—forming process seems to be far greater and it has been attracting a growing attention of many soil scientists particularly in the last decade.

DeLong and Schnitzer [27, 85 - 87] conducted a series of investigations on the role of water soluble organic matter obtained from relatively fresh fallen leaves in the podzol forming process. They found that the most effective fraction was precipitable in a 80 % ethanol solution and that these acidic polysaccharides acted as a protective colloid on an iron oxide.

Bloomfield [6 - 10] also studied the reaction between water extracts of various plant leaves and barks and iron and aluminum, and concluded that the effect of the extracts in solubilizing sesquioxides was due to complex—formation, and that ferric iron was reduced non-microbiologically into ferrous form during the complexation. In his recent study [14, 16, 17] the effective fraction in these processes was thought to be polyphenols.

In addition Bloomfield [13] indicated that the higher-molecular organic matter in the water extracts exerted a dispersing action on a clay suspension, and this was also thought to be due to polyphenolic substances as well as polysaccharides.

Bloomfield [12, 15] succeeded in reproducing a podzol-like profile in a column experiment using a water extract of oak leaves as a leaching agent.

Thenceforth many investigators conducted similar trials to reproduce an artificial profile in a soil column and could obtain podzol-like profiles (Thorp et al. [95], Wright et al. [2, 100], Kawaguchi and Matsuo [44, 46]).

Concerning formation of an illuvial horizon in a podzol profile, Bloomfield [11] showed that adsorption of Fe^{+3}- and/or Al^{+3}-complexes on free sesquioxides and/or clays, and subsequent mineralization of the organic agents causing precipitation of the cations, could be one of the probable mechanisms.

A similar view on the podzolization process to that of Bloomfield was held by Deb [26] and Barshad [3].

Kawaguchi and Matsuo [45] proposed an concept of "quantitative ratio" as a commanding factor in elluviation and illuviation of iron. They thought that reciprocal quantitative relation between mobilizing

agents (some organic matter) and iron oxides to be mobilized governs which of the two processes, elluviation (dissolution) and illuviation (adsorption), takes place, and they proved that the concept was successfully adaptable to their experiments.

At present it is a general understanding that the sifnificance of complex-formation, probably chelation, is of much importance in soil-forming processes. Swindale and Jackson [93] designated a process of dissolution and elluviation caused by chelating agents as "cheluviation" in contrast with the ordinary process caused by leaching water, the later process they called "soluviation".

Chapter 3

THE SOIL HUMUS COMPOSITION OF THE MAIN SOIL GROUPS IN JAPAN

3.1. Introductory

The greater part of the soil humus is considered to be present in association with mineral constituents of the soil, and the status of this association would regulate, on the one hand, the amount and the speed with which mineral nutritional elements are supplied to plants, and, on the other, would exert a great influence on the soil structural conditions.

For this reason there have been accumulated several investigations around the problem in this country. For example, Harada et al. [32 - 34] and Kobo and Fujisawa [48] dealt with so-called clay-humus complexes, and Kosaka and Izeki [55] reported the results of their study on the association of the humus with polyvalent cations. However, all those works were qualitative in their nature and could hardly afford knowledge about the mode of existence of humus in the soil in its entirety.

Tyurin [96] had devised a method to fractionate the soil humus constituents quantitatively according to their association with mineral parts of the soil and more recently Tyurin [97] modified the method in its details. The modified method is being applied widely in the genetic studies of soils in USSR [5, 51, 98].

The author thought that it would be useful to apply this method to the study on the soil humus composition of representative groups of the soils in Japan, and undertook the following experiments.

3.2. Materials

Soils used in this experiment are grouped into four, that is, the upland soil, the dry paddy soil, the wet paddy soil, and the humus volcanic ash soil. The former three and the last one are different in their parent material, that is to say, the former three have developed on materials of Quarternary deposits, while the last mentioned is derived from relatively fresh ejecta of volcanos. The difference among the former three is usually found in their topographic and hydromorphic element, which naturally causes a difference in the land-use. The wet paddy soil is flooded throughout a year and utilized only for the

paddy rice cultivation, while the dry paddy soil has a somewhat deeper ground water table and is flooded only in summer, the rice cultivation period, and drained in the other seasons for cultivation of wheat, barley, and vegetables. The upland soil is naturally never flooded.

Despite that the grouping here brought about is not necessarily genetic in nature and merely conventional, the knowledge we have about the humus in these soils supports the grouping as a reasonable one. As quoted in Chapter 2 Kumada showed that humic acids from various origin are deftly grouped into several types according to many chemical and optical properties, and some of the types correspond to the above soil groups in one to one correspondence.

Location of the soils examined will be given in Table 3.2.

3.3. Methods

i) Tyurin's method of fractionation of soil humus

Tyurin defined four fractions to be separated as follows:

Fraction-1 This fraction is easily dissolved by a dilute alkali solution without any pretreatment of the soil, and represents a polymerization complex of brown humic (or ulmic) and fulvic acids. This complex exists in the soil either in a free state or in a loose combination with aluminum, iron and/or calcium.

Fraction-2 This fraction becomes soluble in a dilute alkali solution only after decalcification of the soil, and represents a polymerization complex of black humic (or true humic) and fulvic acids. This complex exists in the soil in a stable combination with calcium.

Fraction-3 This fraction becomes soluble only after fairly drastic alternate treatments with acid and alkali solutions, and represents a polymerization complex of humic, ulmic and fulvic acids. This complex exists in the soil in a stable combination with sesquioxides.

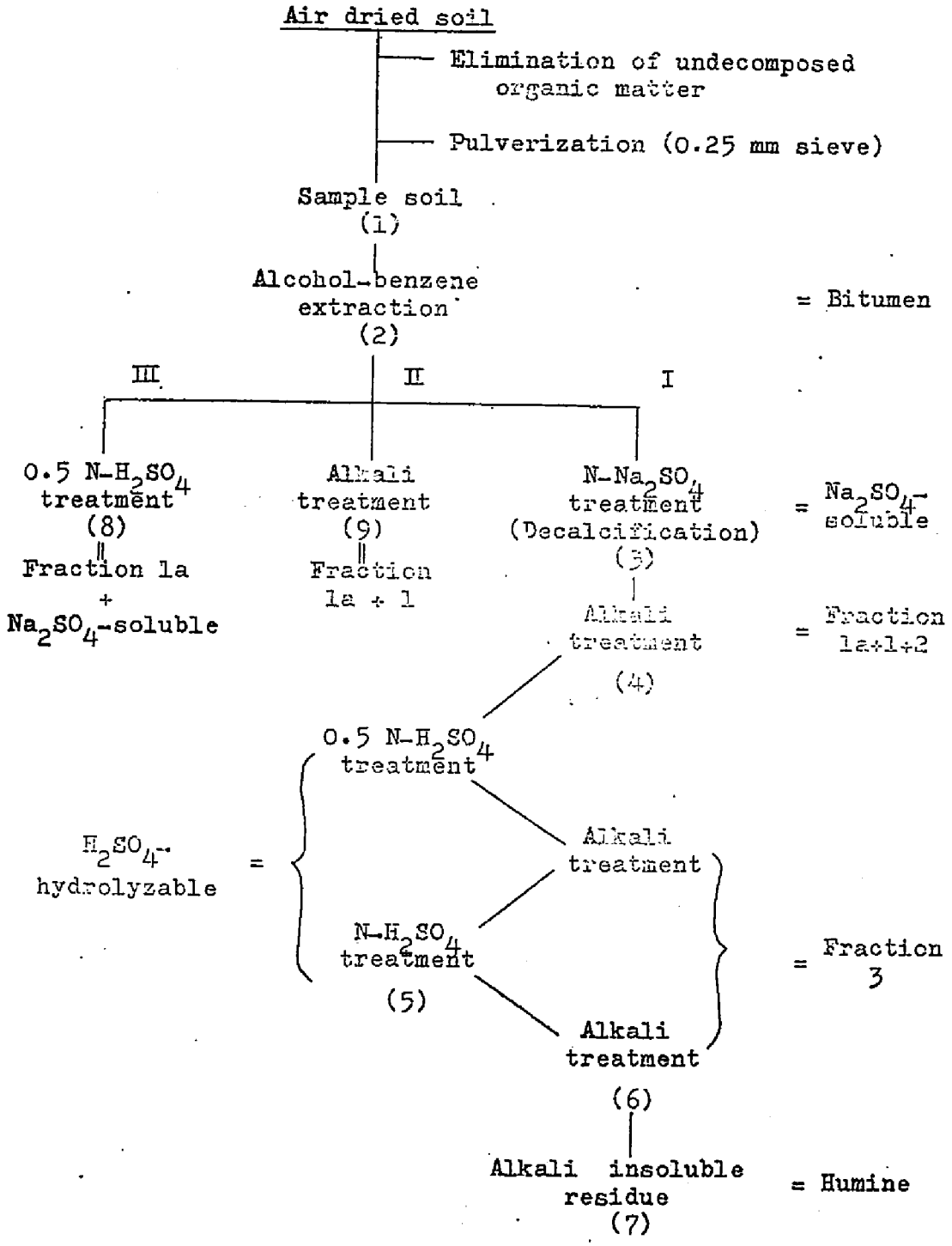
According to Tyurin, these polymerization complexes of the humic and fulvic acids are readily hydrolyzed in the course of dissolution by alkali solutions, and therefore each acid can be determined separately.

Fraction-1a Being most mobile among the fractions, this is easily dissolved by a dilute acid solution at the room temperature, and represents fulvic acids existing either in a free state or in combination

with sesquioxides.

The method of fractionation is summarized as in the following scheme.

The scheme of Tyurin's method



Some notes on the procedures will be given below.

i. Coarse unhumified organic matter must be excluded as thoroughly as possible.

ii. So-called bituminous materials are extracted by alcohol-benzene (1:1) mixture solution in a Soxhlet-apparatus until extracts become colorless. The carbon content of the bitumen is calculated from the dry weight on an assumption that 72 % of the weight is attributable to carbon.

iii. After the extraction of the bitumen, the soil is divided into three parts, and in the courses of I and II (given in the scheme) the sample soil is weighed into centrifugal tubes, in which all the subsequent treatments are carried out.

iv. The treatment with N-Na_2SO_4 solution is conducted for the purpose of decalcification, and three repetitions of this treatment are usually enough for the non-calcareous soils, which represent the greater part of the soils in Japan. The solution obtained from the treatment is made up to a certain volume and analyzed for carbon.

v. The decalcification is followed by the treatment with 0.1 N-NaOH solution. After standing overnight, the alkali solution is separated by centrifugation, when a small volume of the saturated Na_2SO_4 solution is added in order to prevent dispersion of clay. This alkali treatment is repeated until the supernatant solution becomes completely colorless, which needs 6 - 20 repetitions. After the treatment all the supernatant alkali solutions are brought together and made up to a certain volume. A small portion of this is taken for analysis of total carbon. From a greater part of the solution humic acids are precipitated by acidification with H_2SO_4 added to the system so as to give a final concentration of 0.05 N. The precipitate is separated by filtration and then redissolved by 0.02 N-NaOH solution, which is made up to a certain volume and analyzed for the carbon of the humic acids. The carbon of the fulvic acids is known from the difference between the total and the humic acid carbon contents.

vi. After the alkali treatment 0.5 N-H_2SO_4 solution is added and the mixture is stirred. After standing overnight the supernatant is separated by centrifugation. The soil residue is washed with distilled water 2 times. Then the alkali treatment is carried out in the same manner as described above.

Subsequently 1 N-H_2SO_4 solution is added to the soil and the mixture is heated for 1 hour in a boiling water bath. After cooling the supernatant is separated and the soil is washed, and then again the alkali treatment is carried out.

The alkaline supernatants obtained from the two treatments here described are brought together and total as well as humic acid carbon is determined in the same manner as in v.

The two acidic supernatants and the two washings are also brought together and analyzed for carbon. According to Tyurin, the organic materials here determined in the acidic hydrolysates are similar to fulvic acids and are often attributed to hemicelluloses in the soil humus.

vii. The soil residue remaining in the tubes is thoroughly washed with distilled water and then is air-dried in an evaporating dish and finally is weighed. The residual carbon, which is not dissolved, is determined and from this value the content of humine carbon in the original soil is obtained.

viii. In the II course of the scheme, the alkali treatment is carried out for the purpose of determining humic and fulvic carbon of the fraction-1, However, in this determination fulvic acid carbon of the fraction-1a is also involved.

ix. In the III course of the scheme, the fraction-1a is determined by an acid treatment. Usually 10 g of the sample soil is weighed into a flask and 250 ml of a 0.5 N-H_2SO_4 solution is added. After standing overnight the content of the flask is filtered off and a portion of the filtrate is analyzed for carbon. The value here obtained is a sum of the fulvic carbon of the fraction-1a and the Na_2SO_4-soluble carbon. The latter is determined previously in the case of decalcification and must be diminished from the value obtained in the present treatment.

About two weeks are needed for carrying out all the treatments in the scheme. As much as 4 soil samples can be treated simultaneously. Moreover, it is possible to determine nitrogen contents of all the fractions and the sub-fractions, although in our present experiment it is omitted.

ii) Determination of organic carbon

In the case of carbon determination acidic or alkaline sample

solutions are taken in 100 ml Erlenmeyer flasks and preliminarily neutralized by a dilute NaOH or H_2SO_4 solution before evaporating to dryness on a water bath. The carbon in this dried sample is determined by Tyurin's volumetric method. The author used phenylanthranilic acid in the place of diphenylamine as an indicator. The former is more sensitive at the end point and is more conveniently used than the latter (Simakov [88]).

iii) An example of presentation of the results

In Table 3.1. an example of the way of calculation was shown in regard to a soil examined by the author, soil No. 2215. The numbers given in the first column of the table correspond to those given in the scheme of fractionation. As shown in the table the values determined are finally recalculated in order that each value may give percentage of the sum total of them.

3.4. Results and discussions

The results obtained in the present experiment are tabulated in Table 3.2. For purpose of comparison the author quoted a part of the data which Tyurin [98] had obtained in his own experiments in Table 3.3. However, it must be mentioned here that the condition of acidification at the time of precipitation of humic acids was somewhat different in the present experiment from that described above. Tyurin recommended to bring the final sulfuric acid concentration of a system to 0.05 N, while the author added a small excess of a sulfuric acid solution so as to bring the pH of the system to <1 for convenience' sake. Hence, the author's data can be compared with Tyurin's with respect to a total carbon value of a fraction, but not with respect to humic and/or fulvic acid carbon.

Kononova and Bel'chikova [5, 51] found that a ratio of humic acids to fulvic acids shows a characteristic value to each genetic soil type (for example, the ratio is always <1 for podzolic soils and red soils, while it is always >1 for chernozemic soils), and they thought that this would be the most important knowledge drawn from application of this method to the genetic studies of the soil.

The author agrees with them as far as zonal soil types are concerned, in which bioclimatic factors predominate in the process of soil formation. As mentioned in Chapter 2 the author can not draw a

Table 3.1. An example of the method of calculation (Soil No. 2215)

Treatment	Total C		Humic C		Fulvic C	
	% of soil	% of T.C.*	% of soil	% of T.C.*	% of soil	% of T.C.*
(1) Sample soil	5.74	100.0	–	–	–	–
(2) Alcohol-benzene treatment	0.24	4.2	–	–	–	–
(3) N-Na_2SO_4 treatment	0.09	1.6	–	–	–	–
(4) Alkali treatment	2.36	44.6	1.81	31.5	0.75	13.1
(5) H_2SO_4 treatment	0.31	5.4	–	–	–	–
(6) Alkali treatment	0.57	9.9	0.40	7.0	0.17	2.9
(7) Alkali-insoluble residue	2.02	35.2	–	–	–	–
Total	5.79	100.9	2.21	38.5	0.92	16.0
(8) 0.5 N-H_2SO_4 treatment	0.20	3.5	–	–	0.11	1.9
(9) Alkali treatment	2.27	39.6	1.72	29.8	0.55	9.8

	(Analyzed)	(Recalculated)
Bitumen	4.2	4.2
Na_2SO_4-soluble	1.6	1.6
H_2SO_4-hydrolyzable	5.4	5.3
Humic acid	38.5	38.1
Fulvic acid	16.0	15.9
Humine	35.2	34.9
Total	100.9	100.0

Fraction	Humic acid		Fulvic acid	
	(Analyzed)	(Recalculated)	(Analyzed)	(Recalculated)
1a	–	–	1.9	1.9
1	29.8	29.5	7.9	7.8
2	1.7	1.7	3.3	3.3
3	7.0	6.9	2.9	2.9
Total	38.5	38.1	16.0	15.9

* T.C. = Total organic carbon

Table 3.2. - 1 The humus composition of the main soil groups in Japan (% of total organic carbon)

Soils Soil No.	Location	Total carbon (% of soil)	Bitumen	Na_2SO_4 soluble	H_2SO_4-hydrolyzable
UPLAND SOILS					
531	Kyoto, Fushimi	3.16	5.2	1.6	3.6
2005	" , Sakyo	2.99	2.4	1.8	4.2
2599	" , Kamigyo	2.13	2.2	2.2	4.5
DRY PADDY SOILS					
657	Kyoto, Fushimi	1.60	5.2	1.8	5.2
859	" , "	2.42	5.0	1.9	6.0
2169	" , Sakyo	3.46	2.5	1.0	4.1
2171	" , "	1.45	2.1	1.4	7.0
2215	" , "	5.74	4.2	1.6	5.3
Y -1	" , Higashiyama	2.87	3.6	1.7	7.1
I -1	" , Sakyo	3.11	5.8	2.2	7.4
WET PADDY SOILS					
683	Kyoto, Fushimi	4.24	6.9	1.4	4.6
2210	" , Sakyo	2.95	5.5	1.8	4.7
HUMUS VOLCANIC ASH SOILS					
B -1	Shizuoka, Fuji	11.5	1.2	0.7	3.8
B -3	Shimane, Sanbe	9.13	3.4	1.6	3.4
B -4	Kyoto, Komono	12.2	3.0	0.7	2.0
B -5	Kochi, Shimizu	9.04	0.6	0.9	3.0

line strictly between humic and fulvic acids and separation of the one from the other is somewhat conventional. Precipitation of humic acids is conditioned not only by the nature of the humic material but also by pH of the medium as well as kind and amount of cations existing together. This last condition is of particular importance when one deals with intrazonal soils, in which topographic element and/or a parent material plays an important role in soil formation, and this is the case now the author is concerning with. In the volcanic ash soil and the paddy soils aluminum and iron oxides, respectively, exhibit a great activity, and this circumstance would exert an influence on the precipitability of humic acids. This is partly reflected on the data listed in the (A/B) column of Table 3.2., and the author could not find any regularity in the humic acids/fulvic acids ratio.

It would be helpful to compare the data of Table 3.2 with those

Table 3.2. - 2 The humus composition of the main soil groups in Japan (continued) (% of total organic carbon)

Soils Soil No.	Humic acid Fraction 1	2	3	Sum (A)	Fulvic acid Fraction 1a	1	2	3	Sum (B)	A/B	Humine
531	18.7	0	5.5	24.2	6.1	14.9	0.3	4.8	26.1	0.9	39.3
2005	28.6	3.9	5.3	37.8	4.2	13.8	1.7	2.9	22.6	1.7	31.1
2599	21.8	4.4	7.2	33.4	6.4	14.4	1.0	2.3	24.1	1.4	33.5
657	18.5	5.7	5.5	29.7	2.9	10.5	1.2	3.5	18.1	1.7	39.9
859	27.0	2.2	7.7	36.9	2.2	9.6	3.7	1.9	17.4	2.1	32.9
2169	30.9	0	9.9	40.8	4.6	7.5	0	3.4	15.5	2.6	36.2
2171	19.6	6.3	6.3	32.1	4.2	11.9	0.7	4.9	21.7	1.5	35.7
2215	29.5	1.7	6.9	38.1	1.9	7.8	3.3	2.9	15.9	2.4	34.9
Y -1	20.6	3.3	7.4	31.3	2.9	9.8	2.2	4.3	19.2	1.6	37.0
I -1	19.1	3.5	8.3	30.9	2.1	10.2	0	4.6	16.9	1.8	36.8
682	18.1	0	7.3	25.4	4.6	10.6	2.9	3.9	22.0	1.2	29.6
2210	28.4	0	5.8	34.2	2.6	12.0	3.1	3.3	23.0	1.5	30.7
B -1	26.8	0.5	1.3	28.6	15.8	5.5	0.3	6.6	28.1	1.0	37.5
B -3	34.8	1.1	1.1	37.0	12.3	11.1	0	6.9	30.3	1.2	24.4
B -4	47.6	1.2	0.8	49.6	9.5	7.1	0	4.6	21.2	2.3	23.7
B -5	36.0	2.3	1.2	39.5	13.6	6.3	0	7.1	27.0	1.5	29.0

of Table 3.3 for clarifying characteristics of the soils in Japan. The most remarkable feature found in the data of Table 3.2. is that all the soils without exception have a very high content of the fraction-1 (generally above 30 %), and particularly in the humus volcanic ash soils as much as 50 % of the total carbon is present in that fraction.

On the contrary, the fraction-2 is almost negligible in most of the soils used. This general feature seems to be comparable with that of the podzolic soil or the red soil analyzed by Tyurin. However, the author believes, there would be some soils even in this country in which a considerable amount of the fraction-2 does exist, for example, the soils developed on calcareous rocks and those of arable lands having been adequately managed by human activities.

The content of the fraction-3 is about 10 % in all the soils, and it is rather characteristic to the humus volcanic ash soils that the

Table 3.3. The humus composition of the main soil types in USSR (Tyurin, I.V.)

Soil types	Humic acid				Fulvic acid					Humine
	Fraction			Sum	Fraction				Sum	
	1	2	3		1a	1	2	3		
Podsolic soil	17	0	3	20	11	15	0	9	35	32
Slightly podzolized rendzina	7	9	6	22	5	10	6	7	28	40
Degraded chernozem	12	25	7	44	1	5	8	6	20	22
Chernozem	2	20	13	35	0	4	12	6	22	28
Chestnut soil	4	27	6	37	0	3	5	8	16	34
Dark sierozem	5	12	9	26	0	3	5	8	16	43

Note: Figures are percentages on total organic carbon basis.

greater part of the fraction is determined as fulvic acids by the present procedure. The reason for that is not clear yet.

The content of the fraction-1a is greatest in the humus volcanic ash soils and exceeds 10 % of the total carbon, while in the other soils it is relatively scarce and has a tendency to decrease in the order —— the upland soil > the paddy soils. These phenomena suggest, on the one hand, specificity of the humus composition in the humus volcanic ash soils; and, on the other, a greater mobility of some components involved in this fraction.

Insoluble residues, which correspond to "humine" in the general terminology, occupy a considerable percentage of the tatal carbon, ranging from 24 % to 40 %.

Relying on the results mentioned above and taking Tyurin's definition of the fractions into consideration, the author might be able to draw a conclusion that the greater part of the humus found in most of the soils in Japan, or, at least, in the soils examined here, exists either in free state or, more probably, in loose combination with sesquioxides, and not in a stable combination with calcium. This conclusion is coincident with that derived from experiments of other authors [55], and might express one of the most fundamental features attributable to the humus of the soils in Japan. Moreover, the author would have to point out a few specificities in the humus composition of the humus volcanic ash soils, that is, a very high content of the fraction-1 and -1a contrasting to a very low content of the fraction-2 and a high proportion of fulvic acids in the fraction-3 as compared

with the other soil groups. The author infers that these specificities should be closely related to those involved in the nature and the forming processes of the humus volcanic ash soils, and this problem will be dealt with by the author in Chapter 6.

Tyurin's method is really useful for a quantitative evaluation of the humus composition. However, it might be a defect that the method cannot give any precise information on the quality of the humus. The definition of each fraction given by Tyurin involves a little qualitative description of the humus, that is, brown humic or ulmic acids for the fraction-1 and black humic or true humic acids for the fraction-2 are respectively allotted. However, even among the humic acids of the fraction-1 there might be considerable variance and this variance should be adequately informed. For this purpose it would be fitted to measure optical properties of the humic acids of the fraction-1.

Recently Russian investigators [53] held a conference on the methods of humus investigation and reached a similar conclusion to the author's. They recommended to evaluate quality as well as quantity through measurement of optical and colloidal properties. They also proposed that in most of the soils fractionation of bitumen and fraction-3 might be omissible.

3.5. Summary

A method devised by Tyurin for determining soil humus composition was presented and applied to the main soil groups in Japan——the upland soil, the dry paddy soil, the wet paddy soil and the humus volcanic ash soil. Notes on the procedures adopted by the author were given in details.

The main results obtained in the present study are as follows:
1) The greater part of the humus in the soils examined existed mainly in a loose combination with sesquioxides.
2) The humus composition in the humus volcanic ash soil was to some extent specific as compared with the other soil groups and it was infered that the specificities should be closely related to those involved in the genesis of the soil.

It was also pointed out that the lack of information about the quality of the humus would be a defect of Tyurin's method.

Chapter 4

A BONDING MECHANISM BETWEEN THE SOIL HUMUS AND THE POLYVALENT CATIONS PREVAILING IN THE SOIL

4.1. Introductory

In the preceding chapter the author noticed that one of the principal features of the humus in the soils of Japan is that the humus exists in a loose combination with sesquioxides.

In fact, iron and aluminum are the most prevailing polyvalent cations in the soils in Japan. Owing to predominating precipitation over evapo-transpiration, the soils in this country generally tend to become poor in base status and relatively rich in sesquioxides. Moreover, in the volcanic ash soils exceptionally high content of active alumina is often found under the influence of the parent material, while in the paddy fields iron oxides obtain a high activity during the cycle of oxidation and reduction.

Thus, one can easily understand the importance of iron and aluminum in the process of humus-formation and -accumulation. In this chapter the author intends to elucidate a bonding mechanism between soil humus and these polyvalent cations. As reviewed in Chapter 2, studies of complex-formation in the soil have been focused on the problem of minor elements of the first transition series, while behavior of iron and aluminum have been very rarely studied and that the knowledge obtained up to the present has not necessarily been certain.

The author will also examine behavior of calcium, which is considered to play an important role in some calcareous soils.

4.2. Materials

As the sources of humus preparations to be used, the unplowed surface layers of a humus volcanic ash soil (Komeno, Kyoto Pref., soil No. B -4) and of a calcareous soil (Mt. Buko, Chichibu district, Saitama Pref.) were chosen for the reason that aluminum and calcium are supposed to act as stabilizing agents of the humus respectively in the former and in the latter. Location and some other descriptions of the sample soils are as follows:

	Komono soil	Chichibu soil
General description	A humus volcanic ash soil; allitic, humus rich, and considerably acidic in nature.	A calcareous soil developed on limestones; fairly humus rich and neutral in nature.
Latitude (North)	35°10'	35°57'
Longitude (East)	135°25'	139°06'
Altitude	ca. 200 m	ca. 550 m
Mean annual temp.	ca. 14°C	13.5°C*
Precipitation per annum	ca. 1600 mm	1287 mm*
Vegetation	Bamboo grasses, Broad-leaved shrubs, Pine trees.	Zebra grasses, Deciduous broad-leaved trees.

* The data were obtained at the nearest meteorological station (Kumagaya, Saitama Pref.), altitude of which is about 40 m.

4.3. Methods

i) Method of preparation of humus samples

The sample soils were extracted with a 0.15 M-sodium pyrophosphate solution (pH = about 9.5) and an aliquot of the humus extract thus obtained was ultra-filtered through a collodion membrane to make as free from peptized clay particles as possible, and then treated with ion exchange resin columns as explained below. The humus solutions thus prepared were designated respectively as K-W and C-W according to the initial letter of the name of the sample soils. The rest of the humus extract was divided into humic and fulvic acid fractions through acidification with a sulfuric acid solution until pH<1. The humic fractions were reprecipitated three and six times respectively for Chichibu soil and Komono soil and the final precipitates were dissoved into a warm 0.05 N-sodium hydroxide solution. The humic acid solutions were ultra-filtered and finally treated with the ion exchange resin columns and were designated as K-H and C-H respectively. The fulvic fractions were once passed through an active carbon layer and the adsorbed colored substances were eluted by a 0.2 N-sodium hydroxide solution after washing with a dilute acid solution and deionized water. The alkaline eluates were neutralized, dialyzed, evaporated at 35°C to an appropriate volume and finally treated with the ion exchange resin columns.

They are designated respectively as K-F and C-F.

The above-mentioned treatment with the ion exchange resin columns consists of two processes; firstly a humus solution runs through a column of Amberlite IRA-400 resins in OH-form and secondly the leachate from the first process runs through a column of Amberlite IR-120 resins in H-form. The final leachate thus obtained is thought to be an aqueous solution of the humus and despite of its fairly acidic nature (pH 2-3 depending on concentrations of the humus) it remains completely dispersed (cf., Hori and Okuda [39]). The degree of purification of the humus solutions thus prepared is seen in Table 4.1.

ii) Method of titration experiments

A potentiometric titration technique was applied to systems of humus solutions with and without the polyvalent cations.

A humus solution containing 15 mg carbon was taken in a 200 ml beaker and diluted finally up to 70 ml by adding aliquots of a solution of a certain cation, deionized water and a 1 N-KCl solution, which was used to keep the ionic strength constant (μ = 0.1). Each cation was added in two levels refering to results of the column experiments mentioned below, i.e., 5×10^{-5} M and 2×10^{-5} M. The guaranteed reagents of $CaCl_2 \cdot 2H_2O$, $KAl(SO_4)_2 \cdot 12H_2O$, and $NH_4Fe(SO_4)_2 \cdot 12H_2O$ were dissolved in a 0.2 N-HCl solution to prevent precipitation of corresponding hydroxides, and at the time of the use the solutions were neutralized with the equal volume of a 0.2 N-KOH solution so as to result in a KCl concentration of 0.1 N.

The titration was carried out using Mitamura's pH meter connected with an electric stirrer and a microburette, through which a 0.1 N-NaOH solution was added. The pH values were measured with a glass electrode - Ag/AgCl/saturated KCl electrode.

iii) Method of column experiments

Homoionic cation exchange resin columns saturated respectively with Ca^{+2}, Al^{+3} and Fe^{+3} were used for the studies of the reaction between infiltrating humus solutions and these exchangeable cations. The columns used here were identical to those reported by Okuda and Hori [75] and about 10 ml of Amberlite IR-120 resin was filled.

4.4. Results and discussions

i) Results of the titration experiments

Table 4.1. Humus preparations used, their description and purification degree

	Komono soil (T.C.= 12.2%)*	Chichibu soil (T.C.= 6.5 %)*	Description
	K-W	C-W	Extracted by Na-pyrophos. soln., ultra-filtered, I.E.R.-treated.**
	K-H	C-H	Extracted by Na-pyrophos. soln., acid-precipitated, reprecipitated 3-6 times, ultra-filtered, I.E.R.- treated.**
Ca/C $\times 10^3$	8.0	32	
Al/C $\times 10^3$	7.8	2.8	
Fe/C $\times 10^3$	1.1	3.1	
	K-F	C-F	Extracted by Na-pyrophos. soln., acidic filtrate, adsorbed on active carbon, eluted by NaOH soln., neutralized, dialyzed, evaporated (at 35°C), I.E.R.-treated.**
Ca/C $\times 10^3$	9.7	-	
Al/C $\times 10^3$	1.1	-	
Fe/C $\times 10^3$	10	-	

* Total organic carbon, determined by Tyurin's method.

** Ion exchange resin columns.

The titration curves obtained for the humus solutions prepared from Komono soil are illustrated in Figures 4.1. - 4.4. Figure 4.1. shows the titration curves for the 5×10^{-5} M respective cations, and will be compared with those for humus systems illustrated below.

In Figure 4.2. titration curves for K-W systems are presented. In this case 5×10^{-5} M cations were added. The shape of the titration curve for K-W alone is a smooth sigmoid which represents a character of a monobasic acid. Usually, as a titration curve for soil humic acids a polybasic acid-like curve reported by Gillam [31] has been refered to. However, according to a recent paper of Roy [77] one of the features of soil humic acids is considered to be monobasic character appearing in their titration curve. The Roy's finding is fully coincident with that obtained in the present study.

In K-W—Ca^{+2} system, pH decrease and/or humus precipitation was not observed at the time of Ca^{+2} addition, and the titration curve almost coincides with that of the humus alone until a slightly alkaline pH region. In strongly alkaline regions the former curve somewhat deviates from the latter, but the deviation cannot be ascribed to any reaction between the humus and Ca^{+2} when refered to the curve for Ca^{+2}

in Figure 4.1. Thus it might be concluded that calcium ion does not form any complex compounds with the humus solution used.

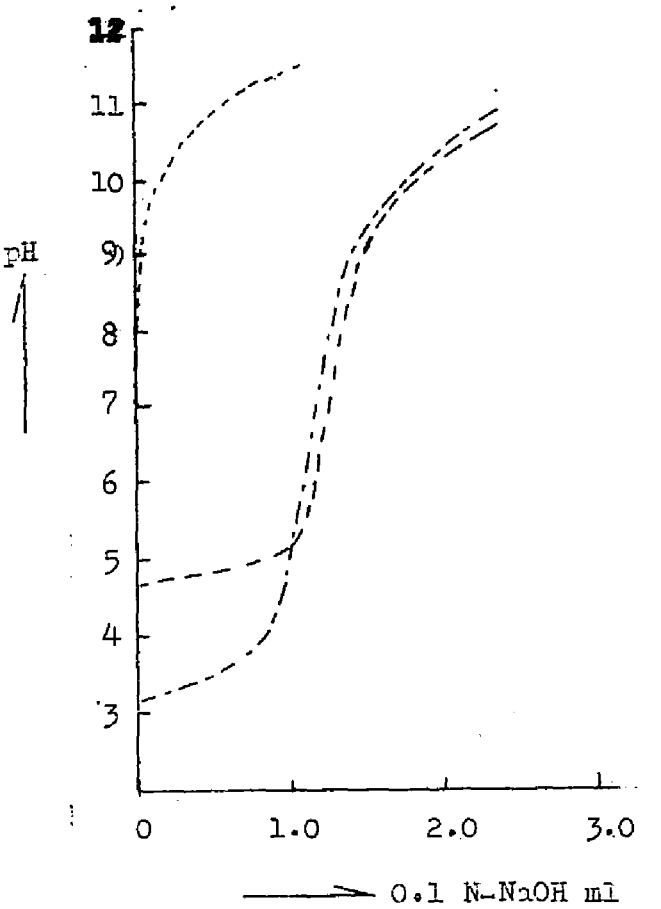


Fig. 4.1. Titration curves of 5×10^{-5} M cations in 70 ml solution; ionic strength, $\mu = 0.1$

In K-W——Al^{+3} system, both pH decrease and humus precipitation always occurred on adding the cation. As the titration proceeded the system showed fairly strong buffering action in the lower pH region, but any inflection of the curve corresponding to the precipitation of aluminum hydroxide (pH = 5, see Fig. 4.1.) was not found. On further additon of the alkali the precipitates were markedly resolved above pH 7 - 8 region being accompanied by alkali consumption. When the sample solution was treated, immediately after the titration, with the ion exchange resin columns as described above, a part of the humus precipitated in the cation exchange resin column. However, the treatment did not necessarily result in the formation of precipitates depending on an elapsed time after the end of the titration.

All these facts suggest that the precipitation of the humus is caused not only by flocculating action of the polyvalent cation, but also by complex-formation, although the former plays a predominant

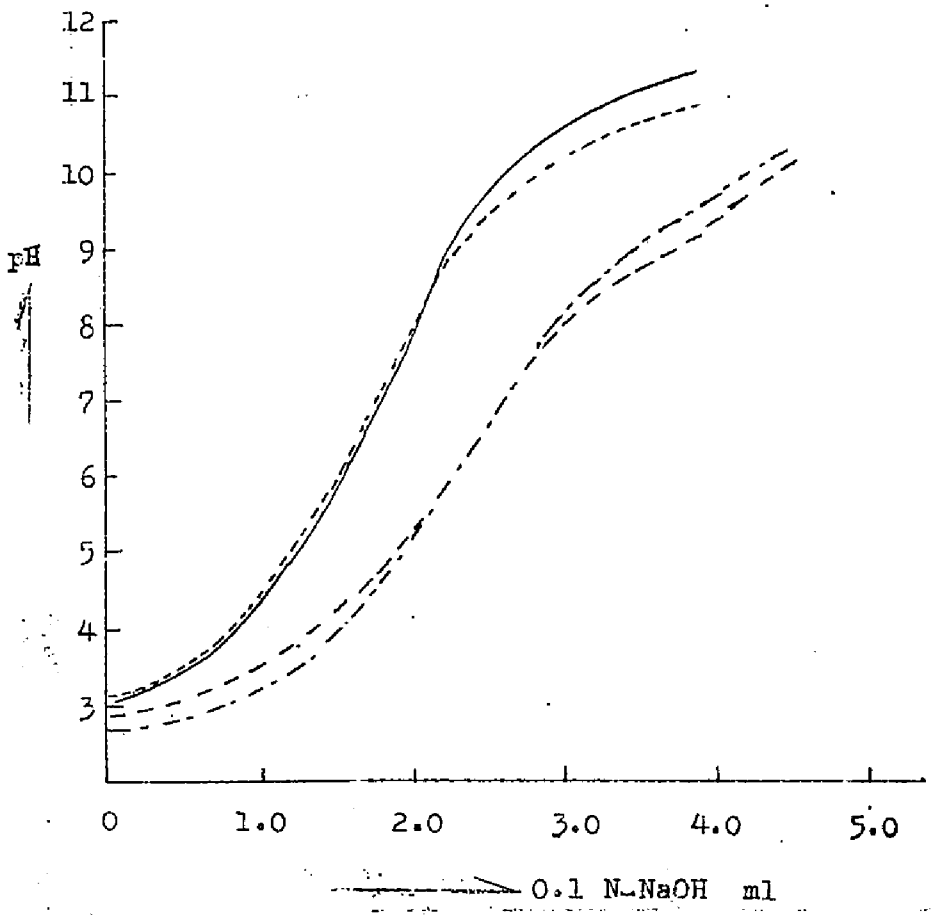


Fig. 4.2. Titration curves of K-W systems (15 mg C/70 ml) with and without 5×10^{-5} M cations; ionic strength, $\mu = 0.1$

———— Humus alone
..... + Ca^{+2}
– – – – – + Al^{+3}
—·—·—·— + Fe^{+3}

role. The precipitates of the complex compounds are subjected to hydrolysis when the medium becomes alkaline and turn into hydroxo-complexes resulting in resolution of the precipitates. Under strongly alkaline conditons the aluminium ion in the complex tends to get rid of the association with humus as an aluminate anion.

In K-W——Fe^{+3} system, pH decrease and humus precipitation occured at the time of Fe^{+3} addition, and the pH decrease was always larger than that found in the Al^{+3} system. This seems to be due to the greater stability of the humus-iron complex than that of the Al^{+3} complex. In the system strong buffering action was displayed , but an inflection of the curve due to precipitation of iron hydroxide (pH = 4, see Fig. 4.1.) was never seen. In a slightly alkaline region the precipitates began to disappear and at the end of the titration no precipitates was found. Iron can be stabilized in an alkaline solution either as a complex compound or as a humus-protected sol. In order to ascertain by which mechanism the iron was stabilized, the same titration technique was applied to a K-W——iron hydroxide sol system. The curve

obtained was almost coincident with that of the humus alone and neither pH decrease nor humus precipitation was observed. Therefore, the re-solution of the precipitates being accompanied by alkali consumption seems to originate from transformation of the humus-iron complex to a hydroxo-complex. Another basis for the inference is that, when the sample solution of the Fe^{+3} system was treated, immediately after the end of the titration, with the ion exchange resin columns, the greater part of the humus precipitated in the cation exchange resin column, while the iron hydroxide sol system did not produce any precipitates. However, even in the Fe^{+3} system, no precipitation occured when a long time (two days in this experiment) had elapsed before the column-treatment. This might be ascribed to dissociation of iron hydroxide from the complex, resulting in the formation of a humus-protected hydroxide sol.

In Figure 4.3. titration curves for K-H systems are presented. The shape of the curves is analogous to that shown in Figure 4.2.in every case examined. However, humic acids in K-H systems were very labile in the solution used and easily flocculated even in the Ca^{+2} system on adding 2×10^{-5} M cation, although pH decrease was not observed. In the case of Fe^{+3} and Al^{+3}, the pH decrease occured and the feature of the curves was similar to that of the corresponding K-W systems.

Figure 4.4. shows the titration curves for K-F systems. In all the systems addition of 5×10^{-5} M cation did not cause precipitation at all. However, with respect to pH decrease and the shape of the curves, the same trend was recognized as found in both K-W and K-H systems. Accordingly, the fulvic acids used are thought to form some water soluble complex compounds with Al^{+3} and Fe^{+3}, but not with Ca^{+2}.

As regards the titration curves for humus preparations alone (in Figures 4.2.- 4.4.), a considerable difference is seen in the amounts of the 0.1 N-NaOH solution required to neutralize them (to pH 7), which are 1.80, 1.65 and 3.60 ml respectively for K-W, K-H and K-F. This point will be discussed later.

For humus solutions prepared from Chichibu soil, analogous results to those of Komono soil were obtained. Therefore, figures for C-W, C-H and C-F systems are omitted. The author anticipated that the humus of a calcareous soil might differ in its reactivity to cations

Fig. 4.3. Titration curves of K-H systems (15 mg C /70 ml) with and without 2×10^{-5} M cations; ionic strength, μ = 0.1

(see right)

——— Humus alone

..... + Ca^{+2}

– – – – – + Al^{+3}

–.–.–.–. + Fe^{+3}

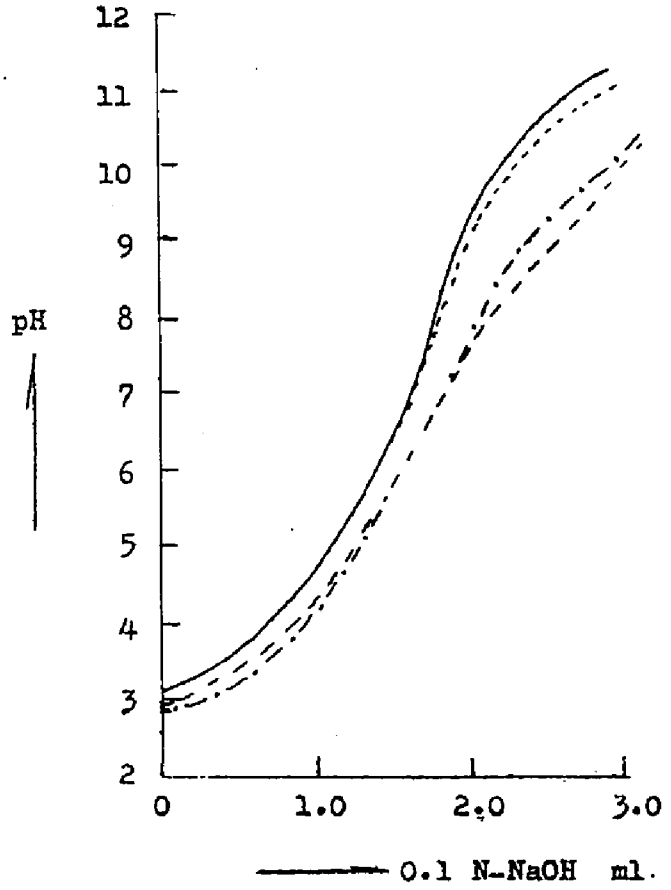
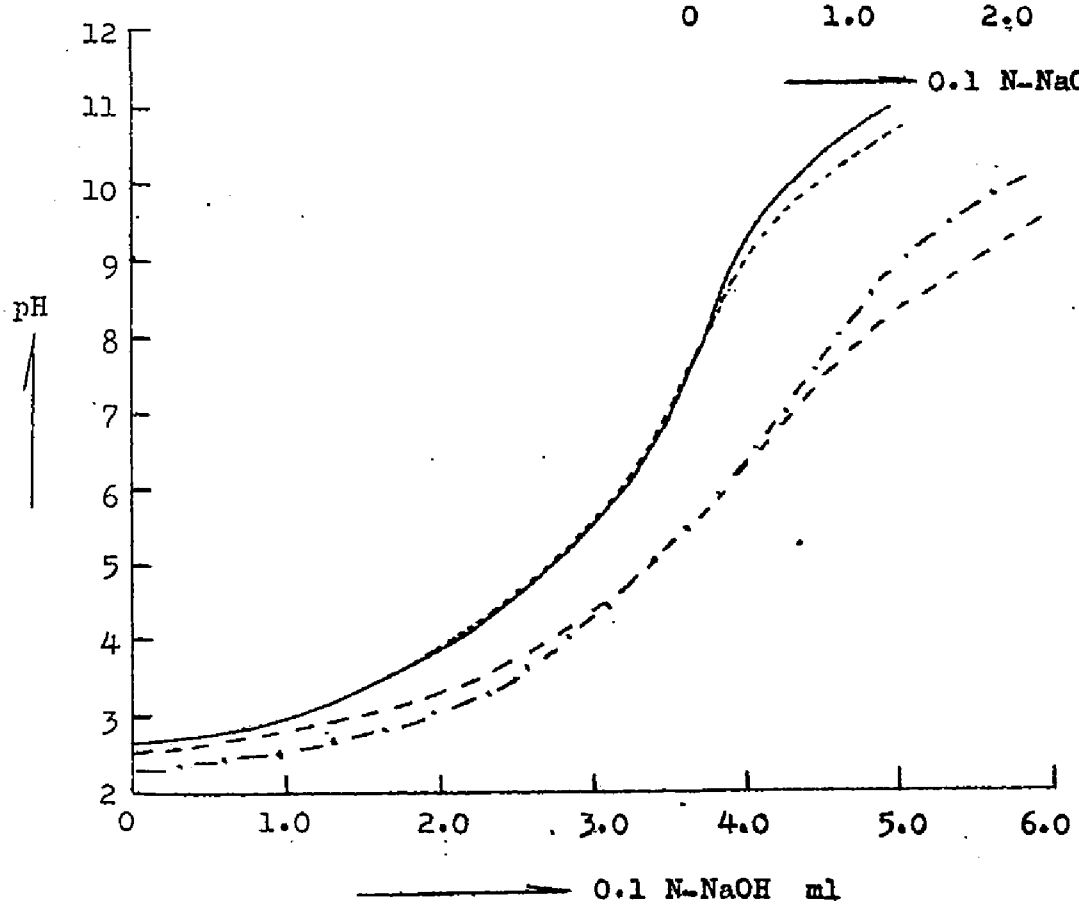


Fig. 4.4. Titration curves of K-F systems (15 mg C /70 ml) with and without 5×10^{-5} M cations; ionic strength, μ = 0.1

(see below)



from that of an allitic Komono soil, but the results obtained in the present study upset his anticipation. Calcium ions do not form any complex compounds with all the humus preparations examined, and on the contrary Al^{+3} and Fe^{+3} ions react with those preparations forming some complexes, which are stable rather in acidic media. However, as shown in Table 4.1., the calcium content of the C-H preparation is considerably high in spite of the elaborate procedures used for purification and this fact suggests that in the processes of humus-formation and -accumulation in the natural Chichibu soil, an intimate association between calcium and humic substances might have been occuring. Further investigations are needed for explaining these processes.

ii) Results of the column experiments

In the first place to clarify specificity involved in the reaction between humus and each polyvalent cation, solubilizing effect of hydrochloric, acetic and humic (K-H) acid solutions, which were adjusted to the same pH, (pH = 3.0), were compared.

As shown in Table 4.2. the acetic acid solution eluted more Ca^{+2} than the HCl solution did and this was due to the buffering action of the former. K-H solution eluted nearly as much Ca^{+2} as the acetic acid did without precipitating in the column. The effect of K-H solution is also ascribed to the buffering action of the humic acids. Fatianov [28], studying the elution effect of apocrenic acids prepared from soil fulvic acids on exchangeable bases in soils, concluded that the buffering action was most respobsible for the effect.

Table 4.2. Quantities of cations eluted from homoionic cation exchange resin columns (IR-120, 10 ml) by hydrochloric, acetic and humic (K-H) acid solutions, which are adjusted to the same pH (pH = 3): Figures are in μM.

Acids	Volume ml	pH	Ca^{+2}	Fe^{+3}	Al^{+3}
Humic (K-H)	50	3.0	77	13	31*
Acetic	50	3.0	75	0.5	27
Hydrochloric	50	3.0	15	tr.	7.4

* A part of the humic acids precipitated in the column, and the datum was obtained for the leachate fraction.

As to Fe^{+3}, the hydrochloric and the acetic acid solutions hardly had solubilizing effect, but the K-H solution eluted far greater amount of Fe^{+3} compared with the former two. This seems to be entirely due to complexing ability of the humus.

Aluminum ions were eluted more readily by the acetic than by the HCl solution. In the case of the K-H solution, although a part of the humic acids precipitated in the column, the leachate fraction contained a larger amount of Al^{+3} than the leachate of the acetic acid solution did. Therefore, here seem to operate both the buffering and the complexing actions of the humic acids.

As deduced from the data in Table 4.2. the bonding force between Fe^{+3} and the resin is considerably stronger than that existing between Al^{+3} and the resin. Hence, it is infered that a part of the humic acids, which has relatively small complexing ability and, therefore, is more susceptible to precipitation as mentioned below, could react and precipitate with Al^{+3} in the column.

Table 4.3. shows quantities and light-absorption properties of precipitates which are formed as a result of reactions between K-H, C-H and K-F and Al^{+3}-column. Naturally, the quantity of precipitates is governed by the concentration of the reactants and, moreover, by the number of repetition of reprecipitation in the course of preparation of the sample humic acids. The data shown in Table 4.3., therefore, must be considered to be merely comparative indexes of relative ease for precipitation of the samples and indexes of relative light-absorbing ability of the fractions both precipitated and leached out. In this experiment a concentration of 29 mg C/80 ml was conveniently used. As shown in the table, quantities of precipitates are nearly equal in K-H and C-H systems but far less in K-F system. E_4 and E_6, extinction coefficients measured at respectively 400 and 600 mμ, and E_4/E_6 values calculated, indexes of the degree of humification, show that the precipitated fraction always has a higher optical density and a more complicated structure than the leachate fraction has.

The comparative data of cation solubilizing abilities of the K-H, C-H and K-F solutions are given in Table 4.4. Preliminary experiments carried out using Al^{+3}-column, showed that the Al/C ratio of the leachate fraction is roughly constant regardless of the concentration of the original sample solutions. Therefore, cation/carbon ratio is

employed here to express the results.

Table 4.3. Quantities and light-absorption properties of humus precipitates in Al^{+3}-column and leachates; the concentration of the sample solutions used is 29 mg C in 80 ml.

Preparations	Fractions	Carbon	E_4*	E_6*	E_4/E_6
		mg			
K-H	Precipitate	13.0	0.85	0.23	3.7
	Leachate	15.8	0.84	0.21	4.0
C-H	Precipitate	14.4	0.59	0.14	4.1
	Leachate	14.7	0.50	0.11	4.5
K-F	Precipitate	4.3	0.47	0.07	6.7
	Leachate	24.2	0.25	0.02	12.5

* E_4 and E_6 values are extinction coefficients measured at 400 and 600 mμ in 0.025 mg C/ml solutions.

The data indicate that the solubilizing ability of K-H and C-H is nearly equal (as seen in the case of Al^{+3}), but fulvic acids (K-F) have considerably higher ability to elute any cation examined than the humic acids have.

Table 4.4. Cation eluting ability of humus preparations from homoionic cation exchange resin columns (IR-120, 10 ml)

Preparations	Ca/C $\times 10^3$	Fe/C $\times 10^3$	Al/C $\times 10^3$
K-H	152	34	52
C-H	-	-	55
K-F	267	77	119

iii) Discussions

The method of preparation of an aqueous humus solution through the treatment with the ion exchange resin columns is of much interest in considering precipitability of the so-called humic acids. Okuda and Hori [74] previously criticized the Simon's method for fractionating "true humic acids" from "rotted products" and ascertained that polyvalent cations associated, such as iron and aluminum, greatly affect the precipitability of humic acids.

Larina and Kasatochkin [63] studied the coagulation of coal humic

acids caused by electrolytes in an alkaline medium using an X-ray analysis and an infra-red spectrophotometry. They, presupposing the model structure given in Chapter 2, considered that, stable existence of the humic acids in an alkaline solution is due to the presence of enough active hydrophilic groups in the peripheral chains, and interaction of the humic acids with polyvalent cations such as Ba^{+2} and Ca^{+2} decreases the stability as a result of complex-formation shown schematically below and brings on precipitation of the huric acids.

$$R(COO)^{-}_{n} + Ca^{+2} \longrightarrow R(COO)^{-}_{n-1}\,COOCa^{+}$$

$$R(COO)^{-}_{n-1}\,COOCa^{+} + R(COO)^{-}_{n} \longrightarrow R(COO)^{-}_{n-1}COOCaCOO(COO)^{-}_{n-1}R$$

They also found that the humic acids obtained from a highly carbonized coal are more susceptible to precipitation owing to development of the hydrophobic aromatic nucleus and consequently decrease in the hydrophilic groups.

A similar inference might be possible from the results obtained in the present study. When Al^{+3} or Fe^{+3} is added to the humic acid solution, the resulting precipitation is chiefly ascribed to the flocculation of the humus particles under the influence of the electrolytes added. However, the pH decrease on adding the cation, the progress of the titration curve and/or the result of the treatment with the ion exchange resins suggests that, at least, a part of the precipitation is due to the complex-formation. It is generally known that many water soluble chelating materials precipitate at the time of chelation, being masked their hydrophilic groups, and the phenomenon is taken as an evidence of the very chelation by Martell and Calvin[66].

The humus, having many functional groups in its molecule, is thought to be a sort of polydentate chelating agents and is possible to form inner-molecular complexes (that is to say chelates), though Larina and Kasatochkin supposed a complex participating two humic acid molecules after the manner of the above-mentioned formula.

Both the iron- and aluminum-humus chelates are stable in acidic media, but become gradually unstable in neutral to alkaline pH region and transform into hydroxo-chelates as a result of a partial hydrolysis, when a part of the hydrophilic groups is released and thus the humic complexes become to be able to exist in the solution. With the increasing alkalinity of media, the hydrolysis proceeds and finally

iron and aluminum are freed from the complex respectively as a hydroxide sol and an aluminate anion.

In fulvic acids——Al^{+3} or -Fe^{+3} system, althought the pH decrease and the feature of the titration curve indicate occurence of chelation, relatively small importance of the hydrophobic aromatic nucleus in the molecule; that is, the predominance of the hydrophilic peripheral chains makes it possible to form water soluble chelates, and precipitation of the fulvic complexes formed does not occure. However, a higher cationic concentration in the medium than that used in the titration experiments may have a possibility to cause precipitation of a part of the fulvic acids which has a relatively developed aromatic nucleus (the experiment quoted in Table 4.3. may be available as an example).

Above-mentioned relations between the structure and the precipitability of a humus molecule are well reflected on the data given in Table 4.3., which indicate that the precipitated fraction has a higher optical density and a more complicated structure than the leachate, the water soluble chelate fraction, has.

Thus, the humic acids, being compared on a basis of carbon content, have less hydrophilic groups, that is, smaller chelating capacity and are more susceptible to precipitation than the fulvic acids, in which both relative importance of the hydrophilic groups and the chelating capacity are high. The explanation given here is fully coincident with the data given in Table 4.4.

It is widely recognized that in the processes of podzol formation the role played by fulvic acids is of much importance [51, 76]. This implies that, the high percentage occupied by fulvic acids in the humus composition of podzolic soils, on the one hand, and the high efficiency which fulvic acids display in migration of soil materials, on the other, are both significant in the podzolization. Table 4.4. can be considered to be an evidence of the high efficiency of fulvic acids.

Finally, association of humus with calcium is to be discussed. In general, calcium has somewhat lower ability to form stable chelates than divalent metal ions of the first transition series have. For example, Broadbent [23] showed that Cu^{+2} has much higher complexing ability than Ca^{+2} has. Larina and Kasatochkin [63], as mentioned above, suggested that some types of complex compounds were formed between humus and Ba^{+2} and/or Ca^{+2}, but in the present study the author did

not obtain any reliable evidence to prove the complex-formation.

However, there exist several soils in nature, in which humus seems to be accumulated under circumstances of abundant presence of calcium ions (e.g., chernozem and rendzina). Therefore, it remains as one of the important problems to clarify the bonding mechanism between the humus and the calcium in those soils.

4.5. Summary

It is an important subject to explain in what mechanism the polyvalent cations such as Ca^{+2}, Al^{+3} and Fe^{+3} associate with the soil humus, because the nature of the association closely relates to the humus accumulation in the soil and also to the role played by the humus in the soil forming processes. Nevertheless, there are relatively few informations directly dealing with the problem, and this is the reason for that the present study was undertaken.

The author carried out several experiments using a potentiometic titration technique and ion exchange resin columns. The results obtained are concisely outlined as follows:

1) Both humic and fulvic acids form complex compounds (probably chelate compounds) with Al^{+3} and/or Fe^{+3}, but not with Ca^{+2}.

2) The humic acid fraction tends to precipitate at the time of chelation and the more susceptible to precipitation the humus is, the more complicated is its structure.

3) The humic acid chelates thus precipitated are partly hydrolyzed in a slightly alkaline medium transforming into water soluble hydroxochelates and in a strongly alkaline medium are completely hydrolyzed resulting in isolation of the cations.

4) The fulvic acid fraction has a higher chelating capacity than has the humic acid and the fulvic acid chelates are usually water soluble.

5) Hence, in the ability to elute the cations from the homoionic cation exchange resin columns a marked difference is recognized between the humic and the fulvic acids, that is, the ability is remarkably higher in the latter than in the former. This fact might suggest that the role played by fulvic acids in soil forming processes, particularly in podzolization, is very much larger than that of humic acids.

Moreover, The author discussed differences in behavior of humic and fulvic acids relying on a schematic model of their structure.

Chapter 5

THE NEUTRALIZATION CAPACITY OF SEVERAL SOIL HUMUS PREPARATIONS —— A DISCUSSION ON THE MOLECULAR STRUCTURE OF THE SOIL HUMUS ——

5.1. Introductory

In the preceding chapter the author has discussed the reactions between the humus and polyvalent cations, especially ferric iron and aluminum, relying on the structural model of humic acids proposed by Russian investigators. As reviewed in Chapter 2, numerous results so far obtained by Kumada [56 - 59] on Japanese soils coincide in many respects with, or do not differ markedly from, those of the Russian workers. However, in the process of the discussion the author has noticed an inconsistency between the supposed structural model and the data of cation exchange capacity obtained by Kumada, who recognized a gradual increase of the capacity along with the progress of humification. On the other hand, Kumada [58] and Kononova [51] reported that sensibility of the humic acids to electrolytes becomes greater as humification proceeds. The sensibility is governed by the relative importance of the hydrophobic nucleus and hydrophilic peripheral chains in the molecule. The explanation is identical to that used in the discussions in Chapter 4.

Then the following relations seem to exist; the progress of humification ——→ increase in the sensibility to electrolytes ——→ depreciation of relative importance of the hydrophilic groups. The principal hydrophilic groups of the humic substances are carboxyl and phenolic hydroxyl groups, which, at the same time, are thought to be responsible for the cation exchange properties exhibited by the humus.

According to the above consideration, the increase in the cation exchange capacity must be incompatible with the decrease of the hydrophilic groups, despite that both the phenomena seem to follow the progress of humification.

Moreover, as mentioned in Chapter 2, according to Kononova [50] and Kobo and Tatsukawa [49] the so-called fulvic acids are analogous in their structure to the less humified humic acids. If humic and fulvic acids are arranged in one and the same series, the fulvic acids must have a structure in which the hydrophilic groups predominate.

This is in accordance with the experimental result which indicates that the amounts of alkali required to neutralize the humus preparations to pH 7 are maximum for the fulvic and minimum for the humic acids (see, Chapter 4).

Hence, the author intended to clarify the apparent discrepancy between the cation exchange property and the supposed structure, and carried out the following experiment.

5.2. Materials

As the source of humic acid preparations the author used the soils listed in Table 5.1. As seen in the table those soils can be grouped into three, that is, the humus volcanic ash soil, the dry paddy soil, and the wet paddy soil, which are considered to be different from each other in their humification degree.(see, Chapter 2).

As samples of fulvic acids K-F and C-F prepared in the preceding experiment were used. They were obtained respectively from Komono and Chichibu soil.

5.3. Methods

Methods of preparation of humic acid samples are same as those adopted in the preceding experiment. Humic acids were extracted by a sodium pyrophosphate solution, precipitated by acidification with a sulfuric acid solution, reprecipitated at least three times, then ultra-filtered and finally treated with ion exchange resin columns.

In order to evaluate the cation exchange capacity of those preparations, the author adopted the neutralization capacity after Gillam [31]. The amount of a 0.1 N-NaOH solution needed for neutralizing them to pH 7 was determined in the same titration technique as that used in the preceding experiment (see Chapter 4), and then the neutralization capacity was calculated on an assumption that the mean carbon contents of the humic acids are 59, 57 and 55 per cent respectively for the humus volcanic ash soil, the dry paddy and the wet paddy soil. For fulvic acid preparations, K-F and C-F, the author assumed that their carbon content is 48 per cent.

5.4. Results and discussions

The results of the experiment are tabulated in Table 5.1. together

Table 5.1. Humification degree (expressed by E_4/E_6), alkali titration value to pH 7 for 15 mg humic and fulvic carbon , and neutralization capacity of various humus preparations

Source of the humus preparations	E_4/E_6 *	0.1 N-NaOH titration value, ml	Neutralization** capacity me/100 g humus
HUMIC ACIDS			
Humus volcanic ash soils			
B -1 Shizuoka, Fuji	3.5	1.65	649
B -4 Kyoto, Komono (K-H)	3.8	1.65	649
B -5 Kochi, Shimizu	4.0	1.49	586
B -3 Shimane, Sanbe	4.1	1.18	464
Dry paddy soils			
2027 Kyoto, Sakyo	5.1	0.95	361
538 " , Fushimi	5.7	0.96	365
65 " , Higashiyama	5.8	0.89	338
I -1 " , Sakyo	5.8	1.22	463
2220 " , "	6.3	1.24	471
Wet paddy soils			
2776 Kyoto, Kamigyo	6.4	0.82	300
95 " , Higashiyama	6.4	1.13	414
2252 " , Sakyo	6.5	0.69	253
851 " , Fushimi	6.6	1.00	367
686 " , "	7.3	1.00	367
FULVIC ACIDS			
K-F (Komono soil, Kyoto)	12.0	3.60	1150
C-F (Chichibu soil, Saitama)	-	3.60	1150

* See the foot note of Table 4.3., in Chapter 4.

** Calculated on an assumption that the mean carbon contents of the humic (obtained from the humus volcanic ash soil, the dry paddy and the wet paddy soil) and fulvic acids are 59, 57, 55 and 48 per cent, respectively.

with the humification degree of the samples.

As shown in Table 5.1., the humification degree represented by E_4/E_6 value is in the following order;

fulvic acids < humic acids of wet paddy soils < humic acids of dry paddy soils < humic acids of humus volcanic ash soils

and the order of the neutralization capacity is different from the above, as follows;

humic acids of wet paddy soils < humic acids of dry paddy soils < humic acids of humus volcanic ash soils < fulvic acids

A part of the result agrees with that of Kumada (i.e., as to the humic acids, gradual increase of the exchange capacity along with the humification degree was ascertained), and another part with that of Kononova and Aleksandrova [52], who reported values of 324 m.e. and 651 m.e. for cation exchange capacities of humic and fulvic acids, respectively, obtained from a strongly podzolized soil. However, as a whole the above mentioned inconsistency was clearly indicated in the data.

Here the author has to examine the idea of the Russian school on the structure of the humic substances.

Kasatochkin and Zilberbrand [43] applied X-ray diffraction and infra-red spectrophotometry to soil humic acids and found that soil humic acids have certain diffraction lines, broad as they are, in about 3.45 Å and 2.4 Å region, which correspond respectively to (002) and (100) reflections of graphite. The presence of these lines, that is, the presence of plane hexagonal network of carbon atoms is available as an immediate experimental indication of a carbonized material. Thus soil humic acids are also shown to be one of the natural carbonized materials. They have another characteristic broad line in 4.6-4.8 Å region, which is usually called γ-band and is thought to be due to peripheral aliphatic groups.

Thus, according to the X-ray diffraction, in humic substances a condensed aromatic nucleus and peripheral aliphatic groups are thought to be present.

IR-spectra of humic substances are useful as another evidence for the presence of both the aromatic nucleus and the aliphatic groups. According to Kasatochkin and Zilberbrand [43] the main absorption bands are as follows:

3.0 μ	OH hydrogen bonded
3.25 μ	C-H aromatic
3.38, 3.42, 3.50 μ	C-H aliphatic
5.8-5.9 μ	C-O in carboxyl
6.2 μ	C-C aromatic
6.6 μ	C-C aromatic
7.9-10.0 μ	C-O oxygen-containing aromatic

The 6.6 μ band is thought to be due to various substituted aromatic rings with aliphatic substitution groups and is clearly seen in less humified humic substances, which have a clear Y-band in their X-ray diffraction pattern.

In accordance with all these results Kasatochkin thought that the model of the structure devised for the carbonized materials (see Fig. 2.1. in Chapter 2) is available also for soil humic substances.

The above-mentioned results and the model proposed by Kasatochkin have been supported by Kononova et al. [50], who studied humic substances of various soils by means of X-ray diffraction and IR-spectrophotometry and obtained the comparable results.

Kumada [58, 59] and Kobo and Tatsukawa [49] also conducted many experiments including the use of X-ray and IR-spectrophotometry in their studies, respectively, on soil humic and fulvic acids of various soils in Japan, and reached a similar view.

In addition to these recent investigations many investigators have been engaged in the rentogenographic studies of soil humic acids since 1938, when Sedletzki and L.Meyer applied the X-ray diffraction to humus for the first time. Some of the investigators identified the graphite-like structure in humic acids and some others could not. However, most of the present humus research workers recognize that humic substances have, at least partly, the graphite-like structure and this structure becomes more and more dominant as humification proceeds.

With respect to constitution of humic acids Thiele [94] thought that the most fundamental part consists of nucleus, bridging and reactive groups. Iso- and/or hetero-cyclic 5 and/or 6 membered ring can be a nucleus and atoms like -O- and -N= and/or atom groups like -NH- and $-CH_2-$ are available as a bridging. Acidic groups like phenolic hydroxyl and carboxyl as well as carbonyl, methoxyl and amino groups are counted as reactive groups. According to Thiele the entire molecule of humic acids consists of many such fundamental parts.

Swaby [91, 92] agrees with other investigators in that humic substances are formed by condensation of polyphenols with nitrogenous compounds. However he thinks that amino acids are only probable nitrogenous compound and these amino acids get into cross linking with polyphenols through their amino group resulting in formation of three

dimensional resinous polymers——humic acids. High cross linking of the numerous phenols, quinones and amino acids gives a heterogeneous molecule without a definite repeating unit and thus makes enzymic breakdown of the product unlikely.

However, Swaby's concept does not contain any idea on the long-term process——humification process——and, moreover, perfectly random structure supposed by him might not be coincident with the result of X-ray diffraction mentioned above. These points seem to be defective in Swaby's concept.

The author has to look into the problem of cation exchange capacity of humic substances. As mentioned above, Kasatochkin's model is not concordant with the fact that cation exchange capacity of humic acids increases with progress of humification.

Scheffer et al. [80, 81] found in their experiments of hydroquinone humic acids that artificial humic acids obtained under the conditions which disturb the progress of humification, for example, restriction of oxidation, show a stronger acidic nature than those humified farther. Scheffer et al. [84] described in their recent monograph of humus that the same tendency is adaptable even to soil humic substances, and that fulvic and brown humic acids are more acidic than those more polymerized, e.g., gray humic acids.

However, as far as the author's experiment is concerned, the view is contrary to the fact. According to Kumada [59], 3.8-3.9 μ absorption band in IR-spectra appears more clearly in the more humified humic acids and the band is thought to be due to O-H stretching of carboxyl group. This fact strongly supports the author's result.

Kononova [54] and Kasatochkin recognized the author's opinion that there exists inconsistency between the model proposed by them and the increasing tendency of cation exchange capacity along with the progress of humification.

Here the author considers on the requisite of a model of humic substances. They show growing hydrophobic property and, at the same time, increasing cation exchange capacity as humification proceeds. How do these two facts coincide with each other ?

The author speculates as follows: The colloidal behavior of humic substances in a solution would be regulated by reciprocal relation between the number of peripheral hydrophilic groups and the dimension of

the molecule, while cation exchange capacity would be determined by the number of dissociable acidic groups in the entire molecule.

As humification proceeds, the number of peripheral hydrophilic groups decreases and the hydrophobic nature of the molecule as a whole is strengthened owing to development of the aromatic nucleus. At the same time the decrease in the peripheral groups would be compensated by the increase in inner-molecular acidic groups, which excert little influence on the colloidal behavior of the molecule, and thus cation exchange capacity would not decrease or rather would increase farther.

The author is not sure whether such a speculation would be probable or not, but he believes that it would be of some use as a working hypothesis for the future investigations.

5.5. Summary

The problem of the structure of humic substances was treated in connection with their exchange property. In the first place an inconsistency between the structural model proposed by the Russian school and the cation exchange property was clearly indicated by measuring neutralization capacity of many humic and fulvic acid preparations.

Then various concepts of the humus structure proposed by the Russian school, Thiele and Swaby were concisely reviewed and the relation between cation exchange capacity and the structure was discussed.

Chapter 6

THE INITIAL STAGE OF ALTERATION OF POLYPHENOLS ADSORBED ON A VOLCANIC ASH MATERIAL

6.1. Introductory

One of the most urgent problems for the Japanese soil scientists is to clarify the genesis of soils developed on volcanic ashes of various origin. These soils cover a considerably wide area of the land surface all over the country, and characterized for the most part by a strongly acidic reaction and the abundant accumulation of humus in the surface horizon. According to Kanno [42], the dominant clay mineral consists of allophane, with varying amounts of gibbsite, hydrous oxides of iron, and hydrated halloysite.

Their taxonomic position is not necessarily definite yet. Kamoshita [41] regards them as a group of the zonal soils and that of brown forest soils, while Kanno [42] believes that they belong to a specific group of the intrazonal soils. American soil scientists designated them as "Ando soils" and this is originally a Japanese name for blackish colored soils. According to Hendricks (see, Aomine and Yoshinaga[1]) soils of this or related types are known to occur widely throughout the Pacific ring of volcanism and in volcanic regions of the East Indies and Africa.

Thus, it is an urgent business to decide an appropriate position of the soils in the general soil classification, and a key to elucidation of the problem, the author believes, is to study the humus-forming and -accumulating processes in the soils.

The humus of these soils has been studied by many investigators. A great many works have been accumulated up to the present and these were once reviewed by the author [62] in another place.

As described in Chapter 2, Kumada [56 - 59] thought that the humic acids of the humus volcanic ash soils are characterized by their high degree of humification and are similar in their general characteristics to those of soils rich in exchangeable calcium such as "Jagaru" in Okinawa. They have a high carbon content, a high cation exchange capacity, a high optical density, a high resistance to oxidative decomposition and a high sensibility to electrolytes in a solution. All

these properties are thought to be characteristic to high humification degree, and hence Kumada drawed the above-mentioned conclusion.

Hayashi [35 - 37] studied the humic acids of the humus volcanic ash soils applying Springer's method [89]. He obtained a humic acid preparation by the same procedure as that usually used for isolation of "gray humic acids", which are dominant in the humus of chernozem. This preparation and the gray humic acids are different from each other in the following points; i. color tone, the former is more blackish than the latter, ii. cations associated, in the former iron and aluminum predominate, while in the latter calcium does, iii. nitrogen content, the former usually has a very low nitrogen as compared to a high carbon content and hence has a very high C/N ratio, iv. acetyl-bromide soluble components, the former shows a relatively high content comparing with the latter. Taking these differences into consideration, Hayashi designated this preparation as "black humic acids" and contrasted this with "gray humic acids".

The black humic acids have somewhat peculiar nature, that is, they are thought to be in a fairly advanced stage of humification, as suggested by Kumada, according to their optical properties, and at the same time they can be considered to be unmatured humic materials according to their high content of the acetyl-bromide soluble components. Hayashi considered that this inconsistent nature of the black humic acids is originated from specific conditions of the volcanic ash soils in which they were formed, for example, a highly acidic reaction and an abnormal microbial environment.

The author, too, considers that environments in which the humus of the volcanic ash soils is formed, are far from those effective for formation of highly humified materials like gray humic acids of chernozem. There must be something specific in the humus-forming and -accumulating processes in the humus volcanic ash soils and it would be reflected on the humus composition of them as suggested in Chapter 3.

In this chapter the author intends to find out some specificities involved in the process of adsorption and the initial stage of alteration of polyphenols on a volcanic ash material, "Akaonji", in comparison with some other materials. The author used polyphenols as a substrate in this experiment for the reason that they are now widely recognized to be one of the most essential constitutional element of the

humus, as described in Chapter 2. The experiment given below can be considered to be a model of the processes existing in the natural humus volcanic ash soils.

6.2. Materials

A crude chestnut tannin preparation for the use of hide-tanning was taken as a source of polyphenols. The chestnut tannin is obtained from bark and wood of castanea sativa or dentata and it belongs to the so-called hydrolyzable tannin, most of the members of which (Ellagitannins) are generally thought to be derivatives of pyrogallol, or more accurately, glycosides of gallic acid [72].

The crude preparation usually contains so-called non-tannin fraction and it is known that the greater part of the non-tannin consists of carbohydrates, that is, sugars and uronides. A rough estimate of the preparation indicates that about 70 % of the weight represents tannin and 30 % non-tannin.[73].

The solid materials used here are as follows:

Akaonji (A)—— A glassy volcanic ash material, obtained from an underlayer of a farm at Yoshiwara, Kochi Pref. X-ray and thermal analyses of the clay fraction of the material indicate that allophane is the only dominant component of it. A few analytical data of this material are given in Table 6.1.

Kamigamo (K)—— A residual weathered material of Paleozoic sandstone, obtained from a substratum of a thin pine wood area at Kamigamo Experimental Plantation of Kyoto University, Kyoto. The dominant clay mineral of the material is halloysite, which is somewhat accompanied by 14 Å minerals. A few analytical data of the material are given in Table 6.1.

Tufaceous sand (S)——Pulverized Tertiary tufaceous sand, produced at Akogi, Mie Pref., sold at a market as a polishing sand for the laboratory use. It contains much glassy materials and quarz and is somewhat stained with iron oxides. The sand was cleansed by a 6 N-HCl solution and then repeatedly washed with deionized water so as to become free from Cl^- ions. A few analytical data of the material are given in Table 6.1.

All those materials were passed through a 50 mesh sieve and the finer fraction was used in the experiment.

6.3. Methods

i) Method of incubation

The author prepared a polyphenol-containing substrate similar to Czapek's in its salt composition, which is given below:

Chestnut tannin (crude)	30 g
Sucrose	10 g
$NaNO_3$	3.0 g
K_2HPO_4	1.0 g
$MgSO_4$	0.5 g
KCl	0.5 g
Deionized water	1 liter

As the mixture yielded a little precipitate it was filtered and the filtrate was used as a substrate. The pH value of the substrate was about 4.0.

Each 250 ml portion of the substrate was taken into a 500 ml Erlenmeyer flask and 50 g of each of the soild materials was added to the flask, which was then sterilized in an autoclave with 1 kg/cm^2 pressure for 30 min.

In June 29th, 1960, 1 ml of a dilute suspension of a humus volcanic ash soil (Komono, Kyoto Pref., soil No. B -4) was inoculated to each sterilized flask and thereafter it was left standing in a room temperature (25°C - 35°C) with occasional shaking. After 2, 4, 8, 14 and 22 weeks from the begining of the incubation, every set of flasks containing any one of the above-mentioned three solid materials was taken out and submitted to analyses. For the final period from 14th to 22nd week the flasks were kept in an incubator (30°C).

Growth of moulds was observed with the naked eyes in (S) system

Table 6.1. A few analytical data of the materials used

Materials	Carbon %	Fe_2O_3 %		Al_2O_3 %	
		Mg-reducible *	N/5-HCl soluble	Mg-reducible *	N/5-HCl soluble
Akaonji (A)	1.04	0.65	0.46	12.5	7.45
Kamigamo (K)	0.15	1.74	0.05	1.87	0.32
Sand (S)	0.03	0.04	0.01	0.08	0.01

* Determined by Mg-reduction method (Kawaguchi and Matsuo, 1954).

after 1 week from the begining and in (K) after about 10 days. The mycelia were green and white in color. In (A) system the solid material obtained remarkably black color in a week owing to adsorption of the organic material, but growth of moulds was observed only after 3 weeks. The mycelia were white in color.

As the control the author set two flasks having no solid, and the one was inoculated (Ti) and the other not (T). These were kept for 22 weeks until the end of the whole incubation term.

In the following description length of the incubation term will be expresses by numerals ranging from 0 to 5. Zero means the very begining of the incubation, and figures 1,2,...5 correspond to the incubation term of 2, 4, 8, 14 and 22 weeks respectively. These numerals will be used in combination with symbols, A, K, S, T, and Ti, given above; for example, A3, T5, etc.

ii) Method of analyses

After the end of each incubation term, the content of each flask was divided into two parts, that is, the supernatant solution and the solid residue, by centrifugation at 7500 rpm for 30 min. The supernatant was filtered through Toyo No. 6 filter paper and pH value of the filtrate was measured with Mitamura's glass-electrode pH meter.

The solid residue was once washed with about 200 ml portion of deionized water and after being filtered the washing solution was put together with the supernatant and finally made up to 500 ml (Solution I).

The solid residue remaining after the washing was put in a Petri dish and air-dried as rapidly as possible applying a mild heating (below 50°C).

A portion of the Solution I was taken for determining carbon concentration of the solution. The procedure used was same as that described in Chapter 3.

Another small portion was treated through ion exchange resin columns (I.E.R.) in the same way as in Chapter 4. After the treatment the effluent solution was made up to a certain volume (Solution II) and carbon concentration of this solution was determined.

Another greater portion was taken for obtaining an acid-precipitable material. A 350 - 400 ml portion of the Solution I was taken in a 500 ml beaker and acidified by a N-H_2SO_4 solution until pH value of

the system became below 1. The resulting precipitate was separated through filtration and washed with a small volume of a 0.1 N-H_2SO_4 solution. Then the precipitate was redissolved in a warm 0.1 N-NaOH solution and again precipitated, filtered and washed as before. Finally it was again dissolved in a warm 0.1 N-NaOH solution and treated through I.E.R. The effluent was made up to a certain volume (Solution III) and the carbon concentration was determined.

With respect to the Solutions II and III, UV-absorption spectra were measured ranging from 250 mμ to 400 mμ every 5 mμ interval with Shimadzu QB 50 photoelectric spectrophotometer.

In relation to the Solution III, the neutralization capacity to pH 7 was measured with the titration technique mentioned in Chapter 5.

The rest of the Solution III, was brought to driness under aeration at a temperature below 50°C. The solid preparation thus obtained was submitted to infra-red spectrophotometry.

The IR-spectra were measured with Shimadzu Ar-275 II S infra-red spectrophotometer using NaCl prism, and in KBr pellet.

With respect to the solid residue, after sieving through a 50 mesh sieve the author determined the carbon content of the solid by Tyurin's method with a slight modification.

The adsorbed phase of the organic matter on the solid was extracted by 0.1 M and 0.15 M- Na pyrophosphate solutions successively; with the solid:solution ratio of 1:10, in a 30°C incubator for about 40 hours in both the extraction.

After separation from the solid through filtration both the extracts were brought together and a precipitate was obtained by acidification with 1:4 H_2SO_4 solution. The precipitate was purified and finally underwent the I.E.R. treatment as before. The effluent was centrifuged at 7500 rpm for 25 min. to avoid contamination of clay and the supernatant was made up to a certain volume (Solution IV). The carbon concentration was determined as usual.

In connection with the Solution IV, the UV-absorption spectra and the neutralization capacity were determined. Moreover, the air-dried sample of the Solution IV was submitted to IR-spectrophotometry.

6.4. Results and discussions.

i) Distribution of organic carbon

Distribution of organic carbon among the whole supernatant, the acid-precipitable fraction of the supernatant, and the adsorbed phase on the solid, was calculated and tabulated in Table 6.2. All the figures for the adsorbed carbon are responsible only for the carbon newly adsorbed on the materials, because a correction was made so as to make the organic carbon content of the original materials equal to zero. The most remarkable fact drawn from the table is the exceptionally high figure for the adsorbed carbon quantity in the system containing (A). Of course the figure for adsorbed carbon can not strictly exclude contamination of microbial residues, but the systems containing (K) and (S) is considered to be more contaminated by them (owing to vigorous growth of microbes), and yet they have far less organic carbon than (A) has.

Table 6.2. Carbon distribution (in mg C)

Systems	1 (2 weeks)		2 (4 weeks)		3 (8 weeks)		
	Liquid	Solid	Liquid	Solid	Liquid	Ppt.	Solid
(A)	1380	2000	1220	2145	1130	6	2235
(K)	3600	136	3250	107	2570	224	182
(S)	3860	67	3650	89	2870	191	209

Systems	4 (14 weeks)			5 (22 weeks)		
	Liquid	Ppt.	Solid	Liquid	Ppt.	Solid
(A)	350	0	2485	120	0	2405
(K)	2230	395	311	2270	735	311
(S)	2570	355	270	2550	493	252
(Ti)	-	-	-	2620	465	-

Ppt. = precipitable fraction

Another result deduced from the table is increment of the acid-precipitable fraction in (K) and (S) with lapse of the incubation term. This latter fact is ascribed to chemical and/or microbiological oxidative polymerization of polyphenols. However, in (A), rapid adsorption of the polymerized products on the solid seems to prevent keeping them in the supernatant and yield no precipitates.

ii) Ultra-violet absorption spectrum

Ultra-violet absorption spectra of the desalted supernatant (Solution II) were illustrated in Figures 6.1. - 6.4. The measurement was made in a 0.05 mg C/ml solution.

As seen in Fig. 6.1. the original tannin (TO) shows a clear minimum and a maximum light absorption in 260 mμ and 280 mμ region, respectively, and the maximum absorption peak is considered to be characteristic to most of the tannin solutions.

With respect to Solution II, light absorption in the entire UV-range examined gradually increase and at the same time the above-mentioned peak becomes less and less marked with lapse of the incubation term. These phenomena can be seen even in the case of the control (T5) which is neither added the solid materials nor inoculated, and hence the author infers that these are at least partly due to oxidative polymerization of polyphenols caused by atmospheric oxygen.

However, it is also obvious that the presence of the solid materials and all the changes —— either chemical or microbial —— of the system caused by the addition of the solids exert a great influence on the UV-spectra. This is judged by the following two facts; with respect to the solutions at a same incubation term, i) light absorption coefficient at 400 mμ is always arranged in the order of (A) $>$ (K) $>$ (S), and ii) conspicuousness of the maximum absorption peak in 280 mμ region decreases just in the reverse order , (S) $>$ (K) $>$ (A), and particularly in A5 the peak is minimized as far as it looks like a terrace.

Relative to the precipitable fraction (Solution III), the measurement of UV-spectra was carried out in a 0.01 mg C/ml solution and the absorption curves were given in Fig. 6.5. The characteristic absorption band in 280 mμ region is not so sharp as in the Solution II and there is no clear difference between (K) and (S) systems and also among the samples of various incubation terms. However, the precipitable fraction of A3, which is the sole sample as such for (A) system, shows merely a slight shoulder at about 280 mμ and the shape of its absorption curve differs clearly from those of the other (K) and (S) samples.

With respect to the adsorbed phase on the solid (Solution IV), besides all the A samples only K5 and S5 were examined because the

Fig. 6.1. Ultra-violet absorption spectra of the liquid phase of (T) system (without solid); 0.05 mg C/ml solution, light path 10 mm.

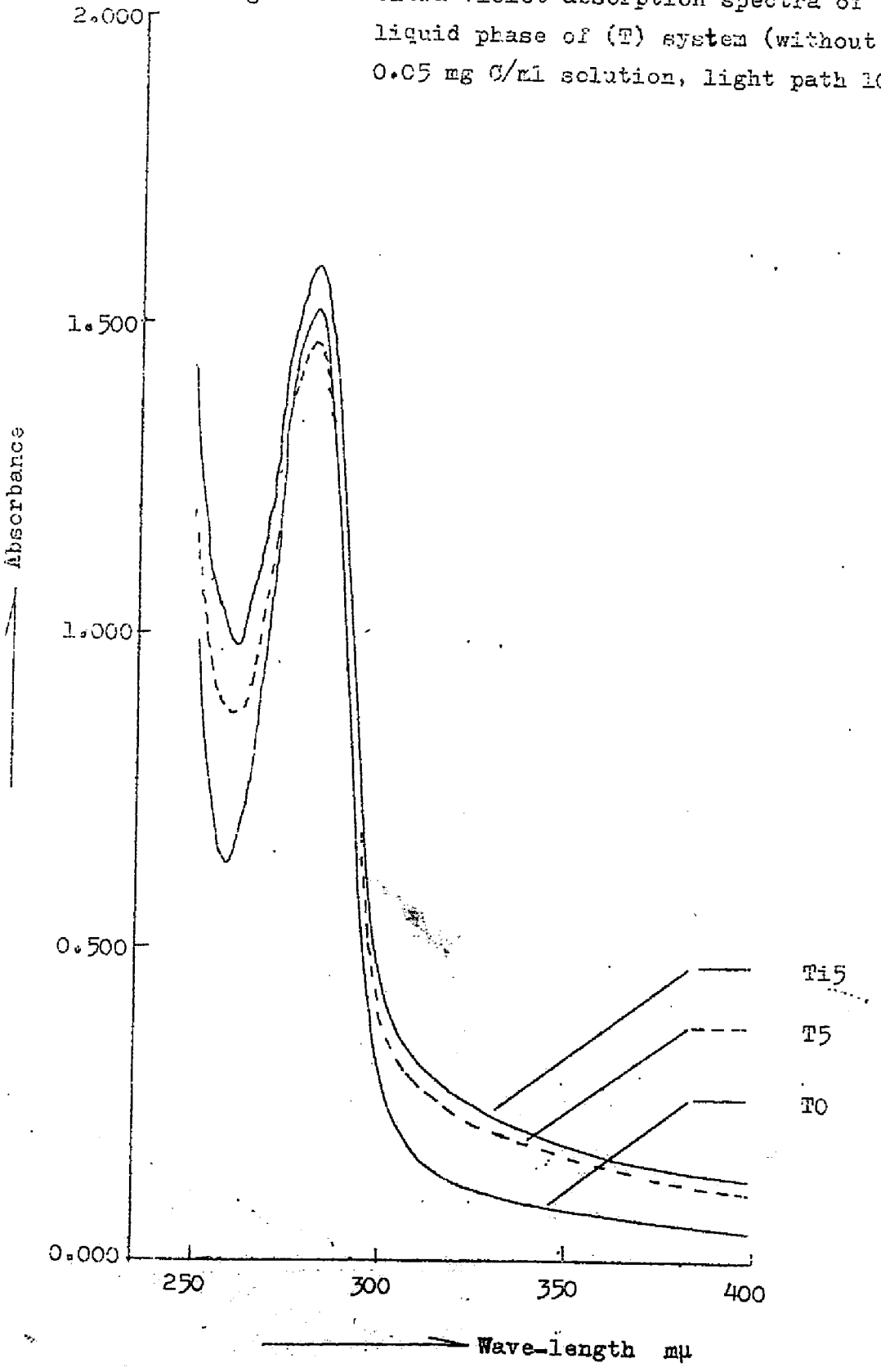


Fig. 6.2. Ultra-violet absorption spectra of the liquid phase of (S) system (Sand); 1.0 0.05 mg C/ml solution, light path 10mm.

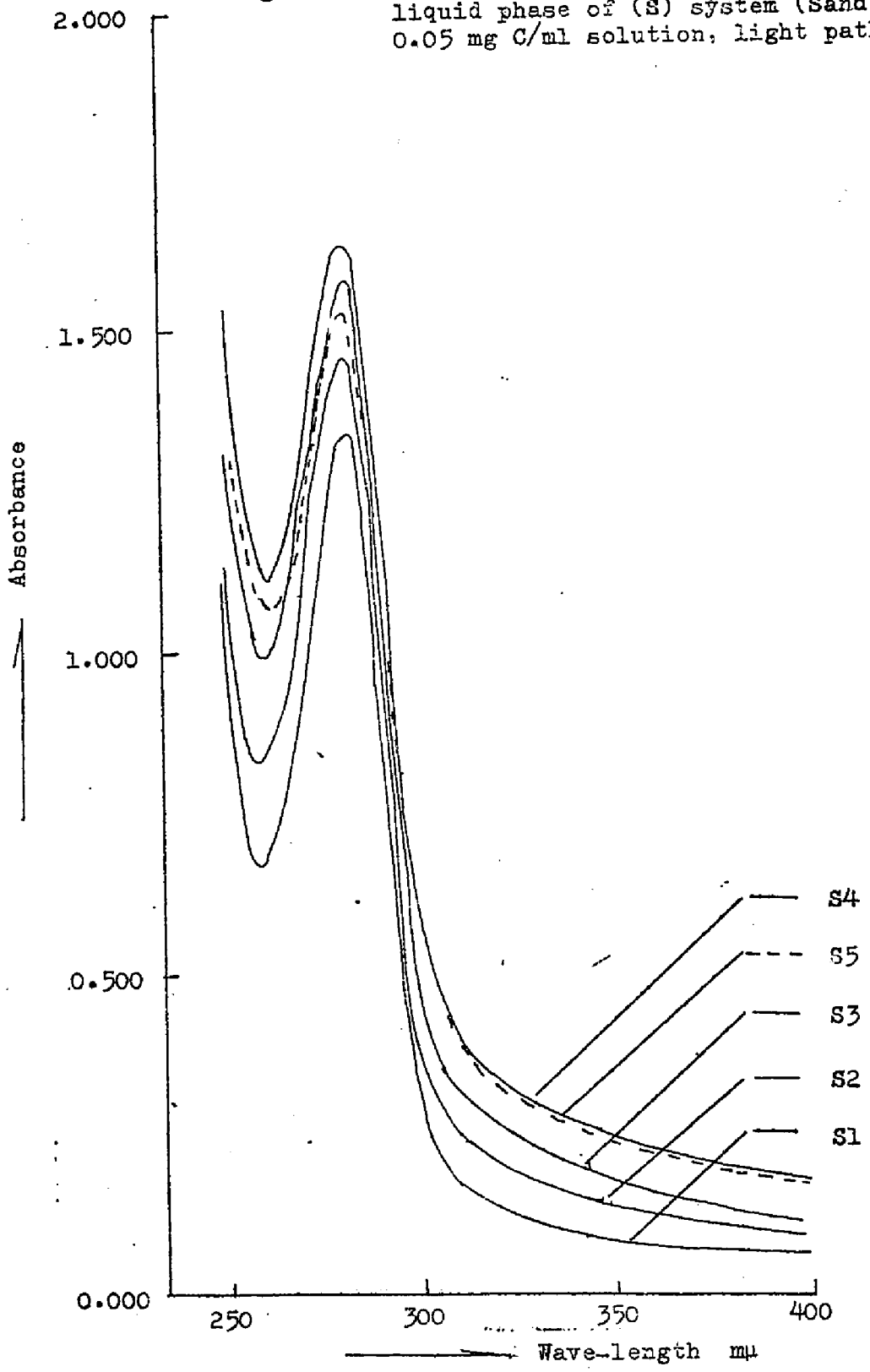


Fig. 6.3. Ultra-violet absorption spectra of the liquid phase of (K) systems (Kamigamo); 0.05 mg C/ml solution, light path 10 mm.

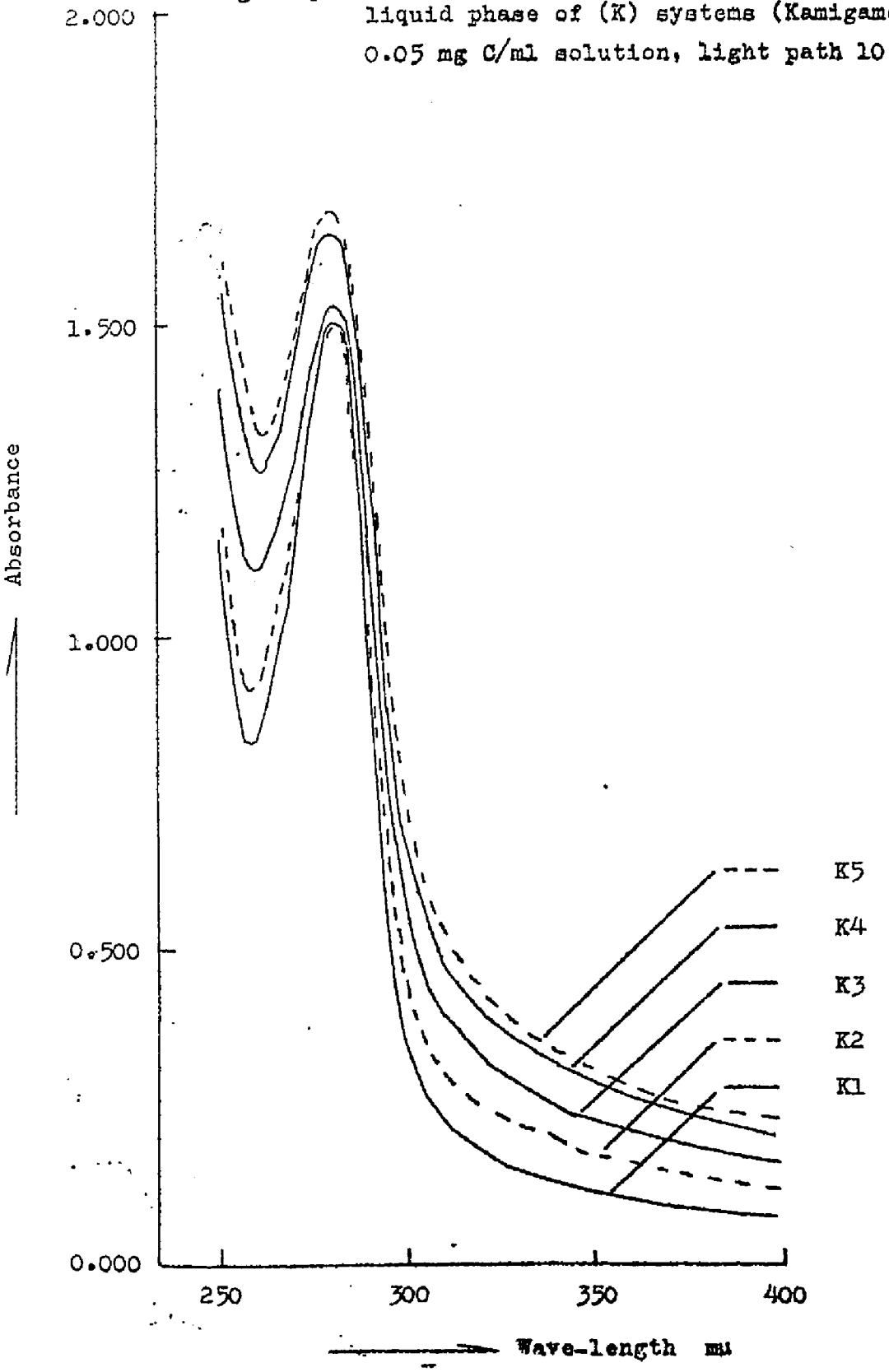
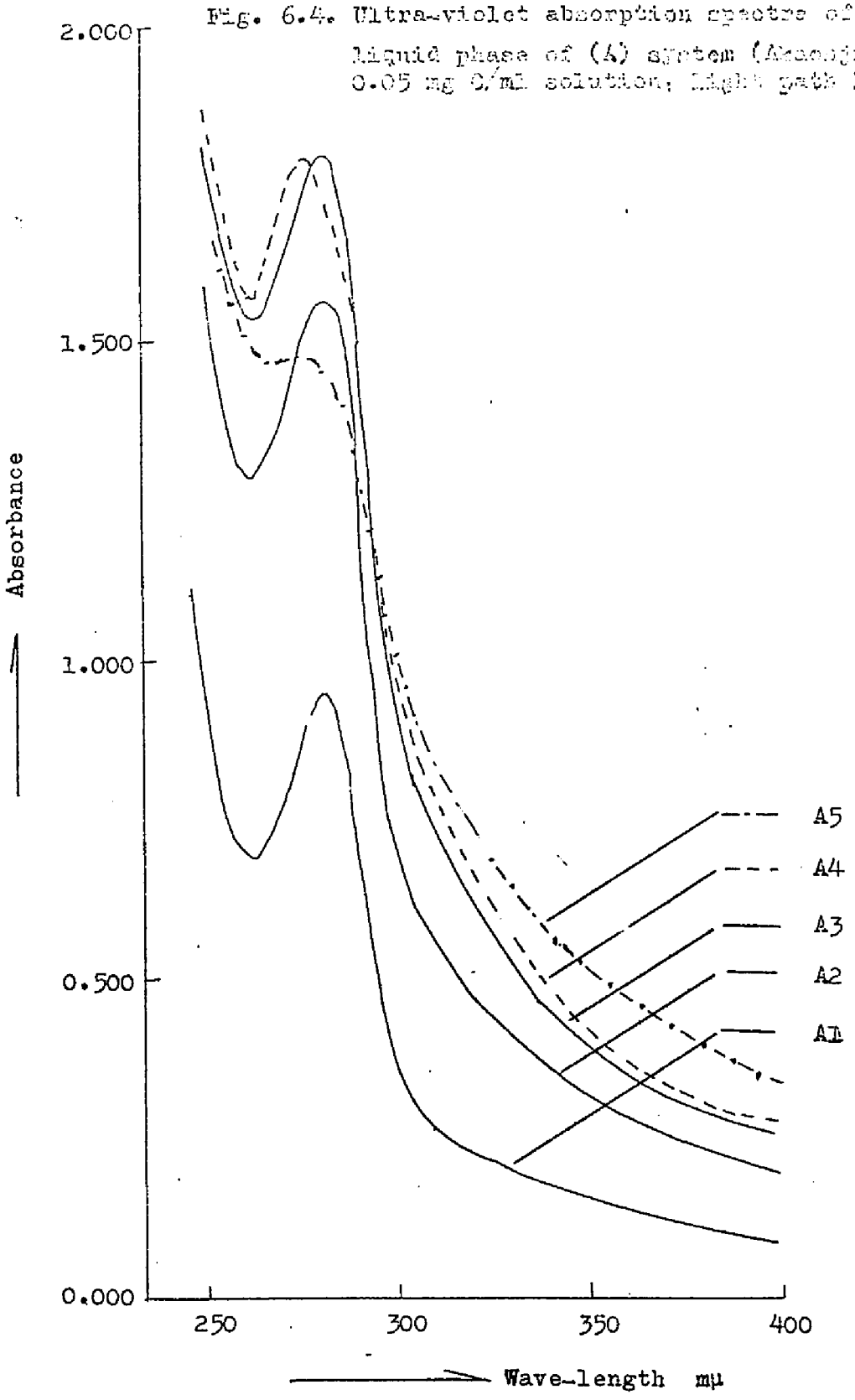


Fig. 6.4. Ultra-violet absorption spectra of the liquid phase of (A) system (Anacin): 0.05 mg %/ml solution; light path 10mm.



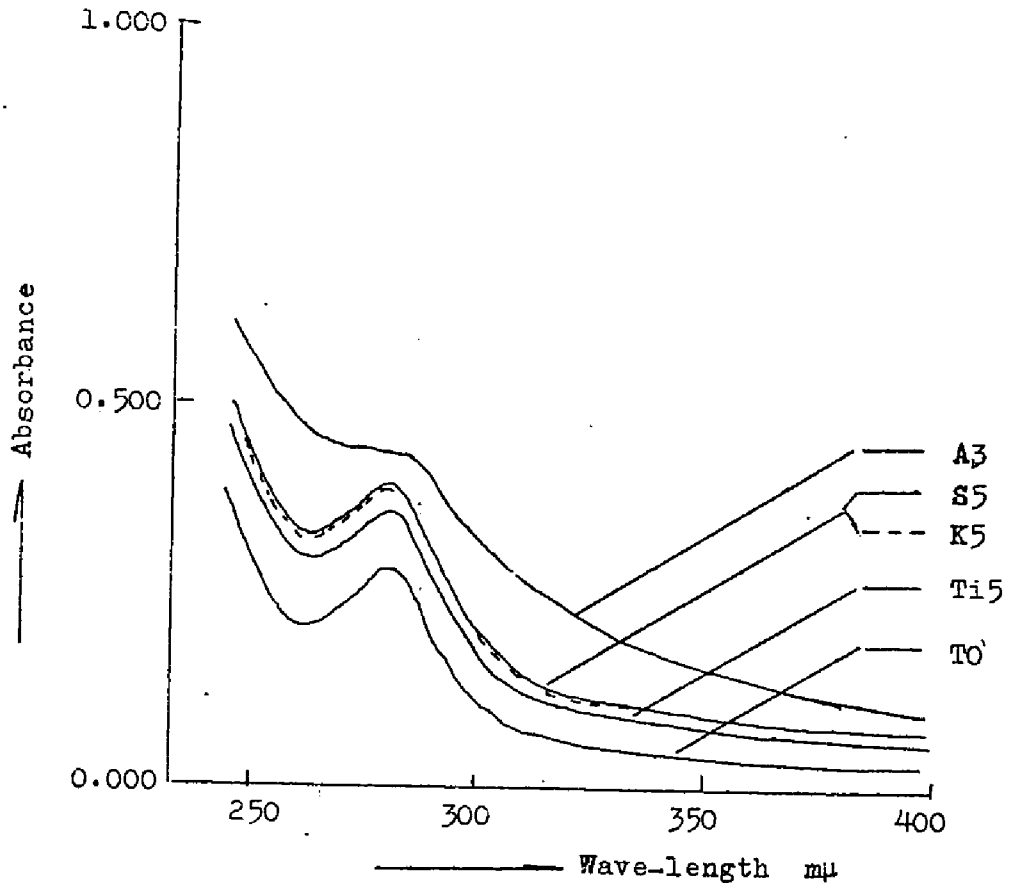


Fig. 6.5. Ultra-violet absorption spectra of the precipitable fraction; 0.01 mg C/ml solution, light path 10 mm.

amount of the adsorbed phase of (K) and (S) systems was very little especially in the earlier samples. The measurement of UV-spectra was carried out in a 0.01 mg C/ ml solution and the absorption curves were illustrated in Fig. 6.6.

UV-spectra of the adsorbed phase of (K) and (S) have a clear terrace-like shoulder in 280 mμ region even after 22 weeks of incubation , while those of (A) show only a slight shoulder already after 2 weeks, when growth of moulds could not be observed with the naked eyes, and remain almost unchanged for the rest of the (A) samples, or rather the light absorption tends to decrease with lapse of the incubation term.

iii) Neutralization capacity

Neutralization capacities of the precipitable fraction (Solution

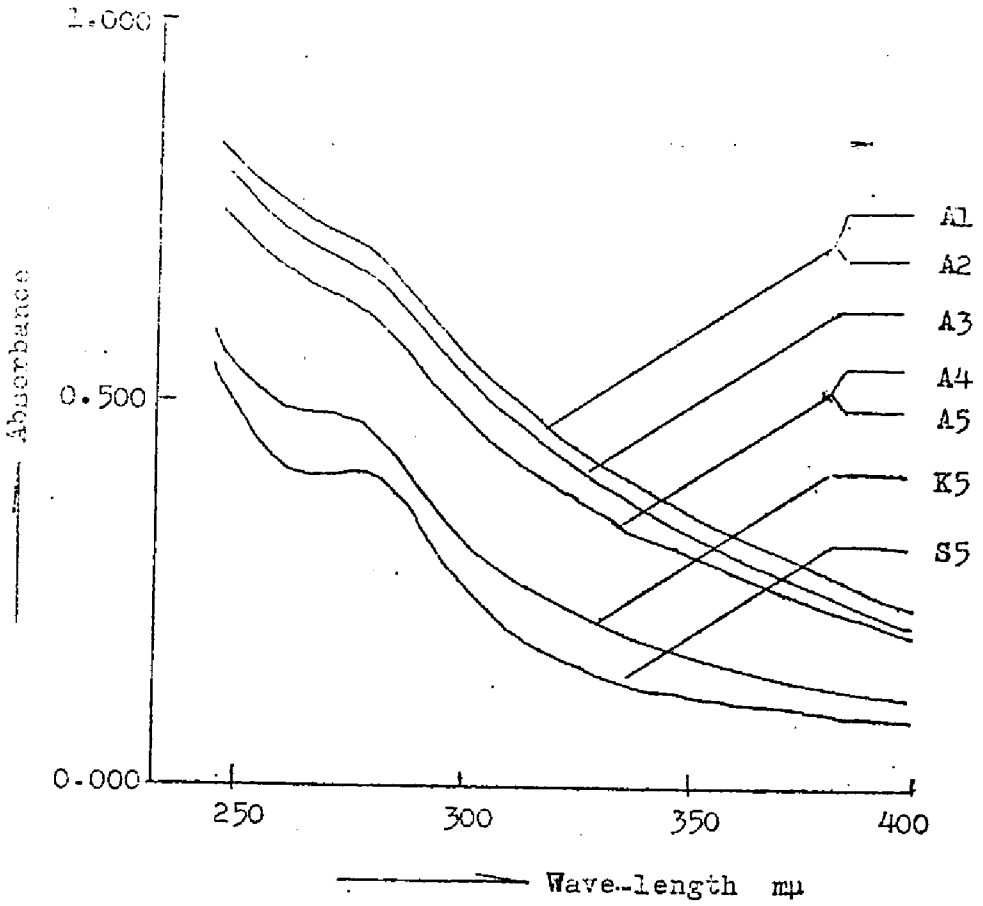
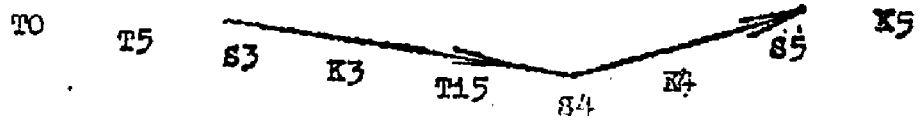


Fig. 6.6. Ultra-violet absorption spectra of the adsorbed phase samples; o.ol mg C/ml solution, light path 10 mm.

III) samples were tabulated in Table 6.3. Titration curves to pH 7 for the preparations obtained from both the original tannin TO and K5 were illustrated in Fig. 6.7. The curve for TO shows an inflection at about pH 7, while that for K5, which has a similar neutralization capacity to TO, does not show any inflections and indicates that some acidic groups having somewhat lower pK values are introduced into the molecule. From these facts it is infered that the main acidic group of TO is phenolic hydroxyl groups, while that of K5 carboxyl.

Taking the characteristics of the titration curves into consideration the author can most reasonably arrange the data for the precipitable fraction in the following order:



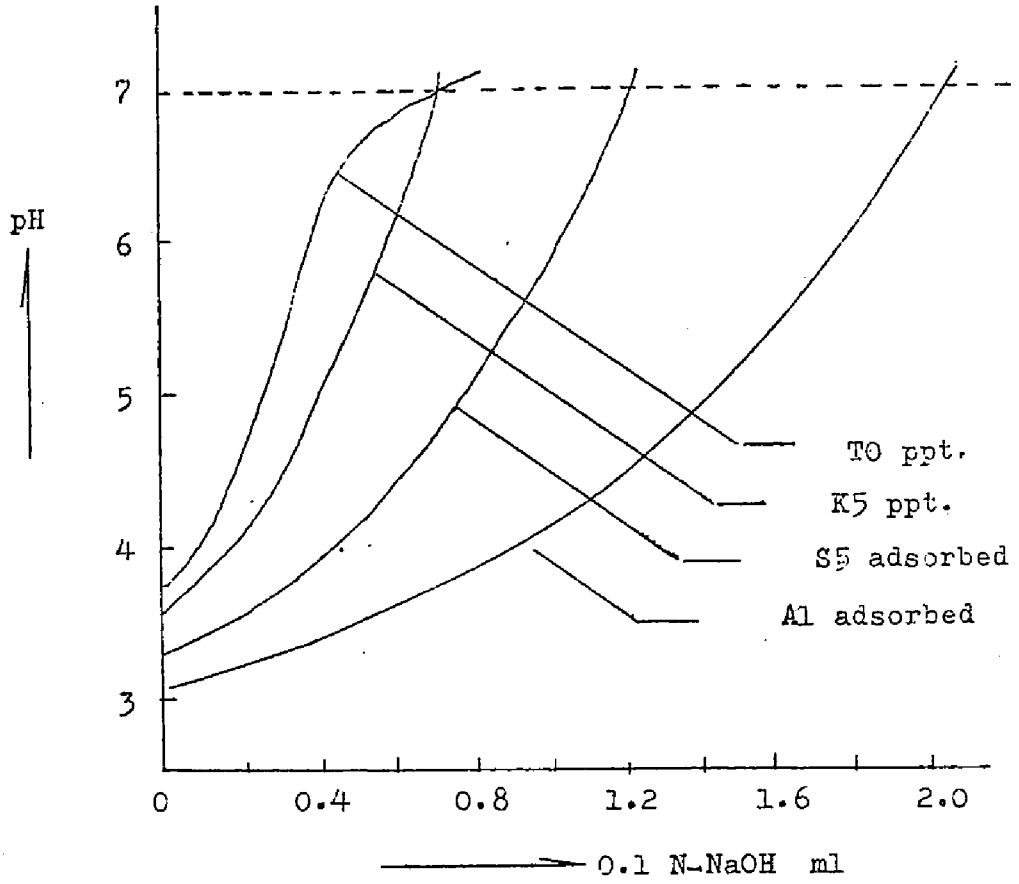


Fig. 6.7. Titration curves of the selected samples of the precipitable fraction and the adsorbed phase in 25mgC/100 ml solution; μ = 0.1

Table 6.3. Neutralization capacities of the precipitable fraction and the adsorbed phase; in ml of 0.1 N-NaOH required for neutralization to pH 7

Precipitable fraction

Systems	Incubation term 0	3	4	5
(T)	0.75	-	-	0.73
(Ti)	-	-	-	0.57
(K)	-	0.64	0.61	0.74
(S)	-	0.72	0.55	0.71

Adsorbed phase

	1	2	3	4	5
(A)	2.08	2.36	2.00	1.90	1.90
(K)	-	-	-	-	1.48
(S)	-	-	-	-	1.26

This arrangement implies two steps: 1) the phenolic hydroxyl groups predominating in the original tannin preparation decrease gradually to a certain level along with the course of alteration of the polyphenols, and then, 2) acidic groups again increase accompanying a change in nature of the group, that is, carboxyl group becomes predominating. This inference is supported by the results of IR-spectrophotometry mentioned below.

With respect to the adsorbed phase, the titration curves for S5 and A1 illustrated also in Fig. 6.7., indicate that their main acidic groups seem to be carboxylic in nature and the neutralization capacities are considerably greater than those obtained for the precipitable fraction. It is particularly noticeable that A1 does not show any inflection due to the presence of phenolic groups and has a very great neutralization capacity. All these changes took place only in two weeks of incubation without active cooperation of microbes.

However, the neutralization capacity does not increase any more with lapse of incubation term, and rather it tends to decrease gradually in a similar way as seen in the case of light absorption of the adsorbed phase of (A) system.

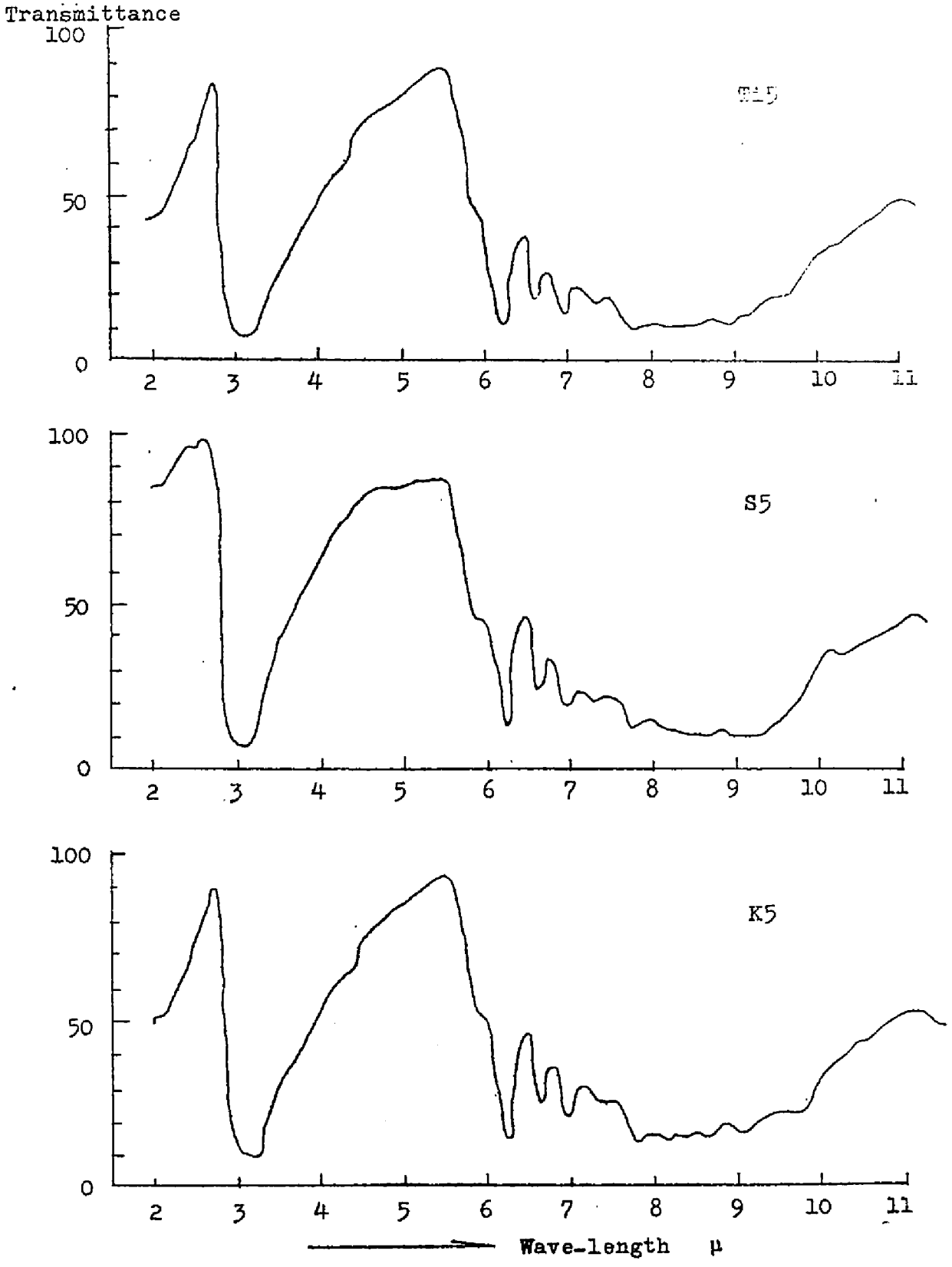
iv) Infra-red absorption spectrum

In Figures 6.8 and 6.9. IR-spectra of some of the preparations obtained from precipitable and adsorbed fractions were illustrated. Spectra of the precipitable fraction of Ti5, S5 and K5 shown in Fig. 6.8. are similar not only to each other but also to the other precipitable samples. They all have several clear absorption bands at 3.1 μ, 6.2 μ, 6.6 μ, 6.9 μ and 7.2-7.4 μ regions, and these bands are thought to be due to the following groups [61]:

3.1 μ	O-H hydrogen-bonded
6.2 μ	C-C double bond of aromatic ring
6.6 μ	C-C double bond of aromatic ring with aliphatic substitution groups
6.9 μ	Aliphatic C-H deformation
7.2-7.4 μ	Aliphatic C-H stretching and phenols

A shoulder found in 5.8-5.9 μ region must be carefully examined in relation to the spectra of adsorbed phase shown in the next figure. It is slightest in Ti5 spectrum and is clearly recognized in K5 and

Fig. 6.8. Infra-red absorption spectra of the precipitable fraction ; NaCl prism, KBr pellet



and S5 spectra. The shoulder seems to be due to C-O stretching.

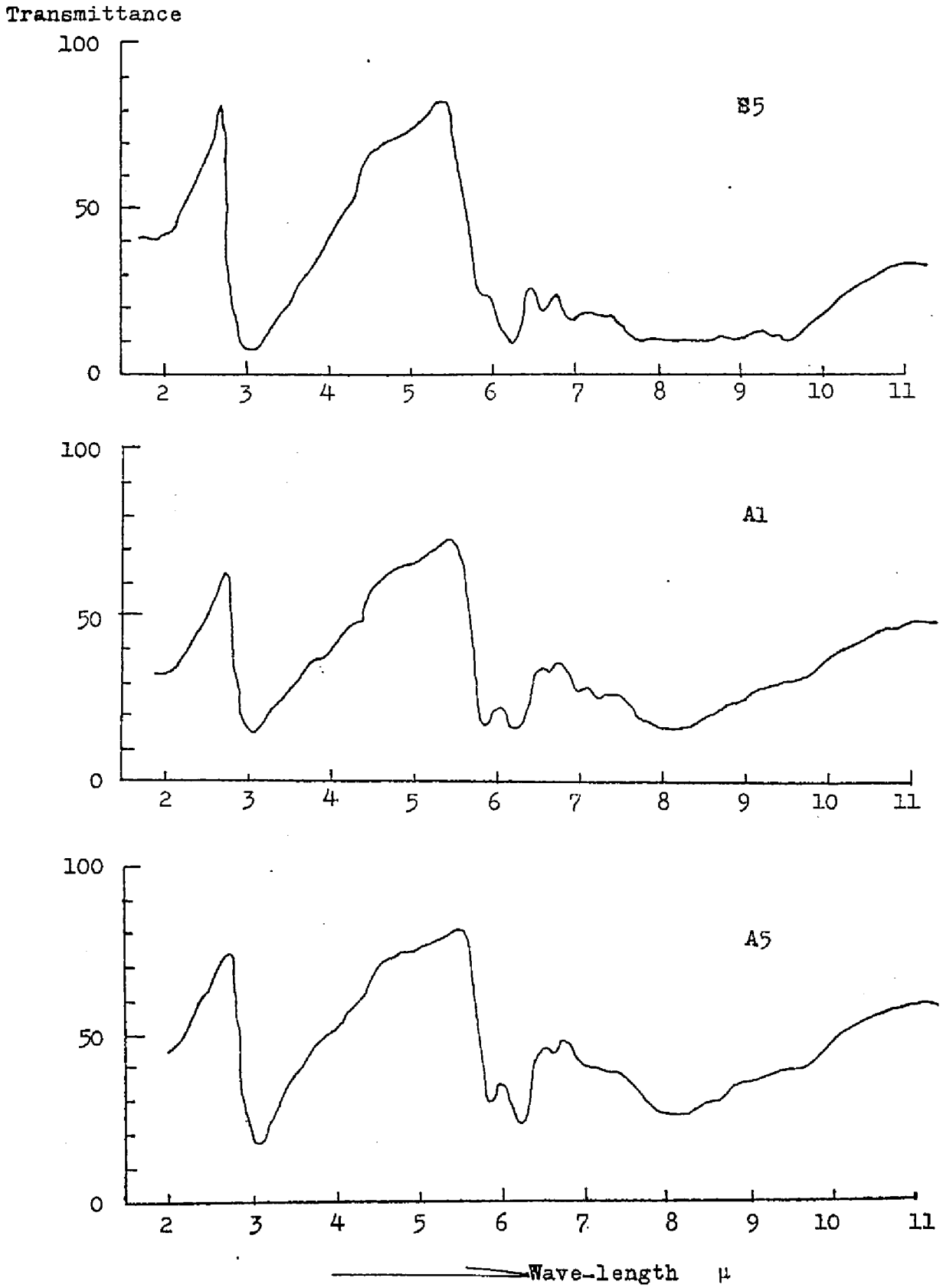
In Fig.6.9. absorption spectra of the adsorbed phase of S5, A1 and A5 were illustrated. The spectrum of K5 (adsorbed) closely resembles to that of S5 and these have somewhat indistinct absorption bands in the region above 6.6 μ although each of the bands found in the spectra of the precipitable fractions are still noticeable.

Increase in intensity of the absorption in 5.8-5.9 μ region, which is due to C-O stretching, is fairly conspicuous. The spectra of A1 and A5 resemble to each other and also to the other adsorbed phase samples of (A) system. These spectra show clear difference from those of precipitable fractions and even from that of S5 (adsorbed) in the general pattern. The 6.6 μ band becomes very weak and 6.9 μ and 7.2-7.4 μ bands become obscure. These phenomena seem to be related to retardation of an aliphatic nature of the adsorbed (A) system samples. However, the most conspicuous feature in the spectra of the adsorbed phase of (A) system is appearance of 5.8-5.9 μ band of high intensity, which is seen as a mere shoulder in the spectra of the precipitable fraction. In connection with this intense 5.8-5.9 μ band, an appearance of a shoulder in 3.8-3.9 μ region is also noteworthy. According to Kumada [59] 3.8-3.9 μ absorption is due to OH in carboxyl group (as mentioned in Chapter 5), so the appearance of this shoulder suggests that the C-O stretching at 5.8-5.9 μ should be originated in carboxyl group. This inference is supported also by the results obtained in the experiment of neutralization capacity., i.e., neutralization capacity of adsorbed (A) system samples was markedly higher than that of the other samples, as mentioned above.

In comparing the spectra of the adsorbed phase of (A) system with those of soil humic acids the author notices resemblance of the general feature of those two kinds of spectra. Some of the absorption bands appearing in the former do not appear in the latter and vice versa, but main bands characteristic to soil humic acids are also present in the spectra of the adsorbed (A) samples.

Especially it is noteworthy that A1 sample shows the resemblance to soil humic acids in its IR-spectrum. It has clear 5.8-5.9 μ band of carboxylic C-O which original tannin scarcely has. It is remarkable that this considerable change could take place in 2 weeks. The results obtained here are fully coincident with the other already mentioned.

Fig. 6.9. Infra-red absorption spectra of the adsorbed phase samples ; NaCl prism, KBr pellet



v) Discussions

From the above-mentioned results it is clearly said that the presence of Akaonji———an allophanic volcanic ash material———not only adsorbs (and stabilizes) extremely much polyphenols but also greatly contributes to acceleration of their oxidative polymerization. UV- and IR-absorption spectra and neutralization capacity certainly supports this deduction.

However, the oxidative polymerization of polyphenols is regulated by the reaction of the medium, so it is necessary to examine this point. In Table 6.4. pH values of the liquid phase of each incubation flask at the time of the end of incubation term were shown. As seen in the table (A) system does not have unusually high reactions throughout the experimental period, and it is concluded that the difference in the reaction does not exert any great influence on the speed of alteration of the polyphenols.

It is also infered that microbial activity would not be effective in acceleration of the alteration process, because it was minimum in (A) system throughout the incubation term as far as observation with the unaided eyes was concerned.

Table 6.4. pH values of the liquid phase

Systems	Incubation term				
	1	2	3	4	5
(A)	4.43	4.45	4.38	4.50	4.70
(K)	3.93	4.27	4.60	4.85	4.68
(S)	4.03	4.30	4.30	5.75	4.45
(Ti)	-	-	-	-	5.25

Then, adsorption-catalystic effect of the very material, Akaonji, comes into question.

Recently Scheffer et al. [82, 83, 103] indicated in their studies of the artificial humic acids that ferric oxides as well as anionic ion exchange resin and silicic acid, can exert a catalytic action on the humification of hydroquinone. They supposed that such a catalytic effect must have an important significance in accumulation and humification of soil organic matter in certain soil types such as pseudo-

chernozem, and distinguished the processes taking place in these soils from those in other zonal soils such as ordinary chernozem, in which humification is primarily due to a microbial process.

The author thinks that the alteration process of polyphenols observed here in the case of Akaonji might be an example of such a catalytic humification process. Polyphenols should undergo an oxidative polymerization while they are co-existing with the allophanic volcanic ash material and are being adsorbed on it, resulting both in disappearance of the characteristic absorption bands of the original tannin and in the increase in the light absorption. At the same time phenolic hydroxyl groups are rapidly replaced by carboxyl groups during the oxidation process and thus the acid groups dissociable in the acidic ($pH < 7$) medium increase. These inferences deftly explain the above-mentioned results.

Then how can these processes take place in the natural volcanic ash soils ? In the first place the reason that the author chose the chestnut tannin as the source of polyphenols should be explained.

First of all the author prefered a natural plant material to purely chemical reagents, because the former surely enters into the soil somewhere on the earth and is submitted to the humification process. Moreover, chestnut tannin is a similar hydrolyzable tannin to that of quercus species [72], which are known to grow on the volcanic ash area as a natural vegetation [101].

According to the recent study conducted by Coulson et al.[24] not only the limited tannin sources but also many other plant species supply polyphenols to the soil both during and after their living period. Polyphenols can be produced through decomposition of lignins and tannins in the soil and are also found in the products of microbial metabolism.

In the allophanic volcanic ash soils all these polyphenols would be adsorbed by the parent material with extremely high adsorptive ability, and once adsorbed they might retrace the oxidative polymerization process catalyzed by the parent material itself.

Of course the role played by microbes in the natural soils is so complex that one can not fully appreciate it, but it is supposed that the microbial activities might be depressed in an inadequate condition such as those found in a strongly acidic medium. However, even in such

an unprofitable medium the above-mentioned catalytic process could be feasible. Thus in the volcanic ash soils having usually a strongly acidic reaction, a lot of humus could be accumulated and the quality o of the humus derived thus from polyphenolic materials should be very advanced in humification degree regardless of the inferior microbial status. However, with respect to plant materials other than polyphenols their humification degree might remain backward owing to the poor microbial activity, and these circumstances would explain the cause of the apparent inconsistency found in the nature of the humic acids mentioned in the very begining of this chapter.

In this sense the humic acids of the volcanic ash soil cannot directly be comparable with those of other normal soils having usual microbial humification processes, though both often have apparent similarities. And this peculiarity is importanct in considering the taxonomic problem of the humus volcanic ash soil.

In the author's opinion this soil cannot be classified as a member of the zonal soils though Kamoshita [41] did so, but rather as an intrazonal soil having a peculiarity in its parent material.

The result obtained in the case of neutralization capacity is interesting in connection with the problem of the structure, especially that of cation exchange groups of the humus.

As discussed in Chapter 5, Scheffer et al. [80 - 82, 84] found the reduction of acidic nature of hydroquinone humic acids along with their humification degree, and even with respect to soil humic acids they hold fast to the same view.

The tendency found in the earlier stage of the alteration of cation exchange property of the polyphenols seems to coincide with Scheffer's view, that is to say, phenolic hydroxyls diminish as a result of condensation of aromatic rings, while that found in the latter stage is concordant with the experimental data given in Chapter 5 and reminds us the increase of the cation exchange capacity of soil humic acids along with the progress of humification process. This increase in the cation exchange capacity might involve introduction of some new acidic groups, presumably carboxyl groups as ascertained in the present investigation.

6.5. Summary

With intention to clarify the peculiarity involved in the humus-forming and -accumulating processes in the humus volcanic ash soil, an investigation of the initial stage of alteration of polyphenols adsorbed on a volcanic ash material was carried out.

Chestnut tannin and Akaonji were used as experimental materials respectively for polyphenols and a volcanic ash material.

The results obtained are concisely summarized as follows:

1) Absorptive capacity of Akaonji for polyphenols was more than ten times as great as that of the other solid materials used for the purpose of comparison.

2) Acid-precipitable fraction was very scarce in Akaonji system throughout the incubation period, while in the other systems it gradually increased with lapse of incubation term.

3) UV-absorption spectra of the original tannin solution shows the minimum and the maximum absorption bands respectively in 260 and 280 mμ regions. The characteristic bands became less and less distinct and light absorption measured at 400 mμ more and more increased as the alteration of polyphenols proceeded. Thus as to the liquid phase, Akaonji sample had the highest optical density and the most indistinct absorption bands among the samples of the same incubation term.

With respect to the acid-precipitable fraction as well as the adsorbed phase of Akaonji system underwent the most conspicuous alteration and had an absorption curve similar to that of soil humic acids. It is worth mentioning that this alteration was attained only in 2 weeks of incubation.

4) The neutralization capacity of the polyphenols decreased at first gradually to a certain level as the alteration proceeded, and then showed a gradual increase. The decrease in the earlier stage seemed to be due to decrease in phenolic hydroxyl groups caused by condensation of the original polyphenols and the increase in the later stage might be ascribed to appearance of carboxyl groups produced through oxidation of polyphenols. The adsorbed phase of Akaonji system showed a high neutralization capacity, and it was also true for the sample incubated only for 2 weeks.

5) IR-spectra of the adsorbed phase of Akaonji system clearly showed 5.8–5.9 μ absorption band due to carboxyl groups, and thus supported

the above-mentioned inference.

6) All these results strongly suggest that Akaonji exerts an adsorption-catalystic effect on the oxidative polymerization of the polyphenols, and this is thought to be one of the most principal features of the volcanic ash soil.

The author pointed out that this peculiarity should be taken into consideration in deciding the taxonomic position of the humus volcanic ash soil.

Chapter 7

A COLUMN EXPERIMENT OF THE EFFECTS OF SOME MOBILE ORGANIC MATERIALS ON THE SOIL-FORMING PROCESS

7.1. Introductory

Besides the role as a skeletal constituent of humus, polyphenols play another important role in the soil-forming process. As mentioned in Chapter 2, recently the studies of leaf leachates as a soil forming agent have been widely undertaken by several investigators. Bloomfield [13, 14, 16, 17] regards polyphenols as the most active component of the leaf leachate materials in mobilizing such soil constituents as iron, aluminum and clay.

However, the role of polyphenols in the soil-forming processes has rarely been studied and is even now somewhat uncertain. Recently Coulson et al. [25] examined D- and Epi-catechin in their model column experiments and found that those catechins caused reduction of ferric iron to ferrous state and thus raised the mobility of iron. However, they confined the role of them only to reduction of iron and could not find any effect on mobilization of aluminum.

The author intended to examine the soil-forming ability of the same tannin preparation as that used in the preceding chapter and also a cmmercial tannic acid in a column experiment. They were chosen partly for the sake of convenience, but partly for the reason that they are sure to get into the soil somewhere on the earth.

Together with those polyphenols the author examined a polyuronic acid sample obtained from a commercial pectin. According to Forsyth [30] and many other investigators [90] polysaccharides including uronides are widely found in the soil and in the soil solution. Especially uronides are thought to be present in the soil relatively abundantly and, moreover, they are supposed to play an important role in soil structure formation. Besides, the author used the polyuronic acid as a sample for the reason that they might have chelating ability, though it is not so strong.

Furthermore, the author intended in this experiment to throw some light on humus-accumulation process along a soil profile with relation to its active oxides content, especially that of active alumina. The

process to be examined below——adsorption of polyphenols on the soil ——may precede those described in Chapter 6——oxidative changes of polyphenols at the surface of the soil particles.

7.2. Materials

i) Column adsorbents

The same three materials as those used in the preceding chapter were used as column adsorbents; Akaonji, Kamigamo and Sand. Carbon and free oxides contents were given in Table 6.1. in Chapter 6.

ii) Leaching solutions

The crude chestnut tannin preparate was once dissolved into a small portion of water and then the solution was shaken with methylethylketone in a separating funnel. The methylethylketone fraction was separated and the solvent was driven off by a vacuum distillation at 40°C. According to Okamura [73] this procedure greatly diminishes the content of non-tannin fraction and the tannin content of the chestnut preparate increased to 93 % from about 70 % of the original crude preparate.

The polyuronic acid preparate was obtained by a partial hydrolysis of a commercial pectin (from citrus). The pectin was boild with a 1:3 HCl solution for a while and the hydrolysate solution was filtered. The absolute ethylalcohol was added to the filtrate so as to make the alcohol concentration above 70 %. Precipitates were gathered and washed with alcohol through centrifugation. The precipitates thus obtained are considered to be relatively low molecular polygalacturonic acids and soluble in cold water.

Besides the purified chestnut tannin and the polyuronic acid preparates, a commercial tannic acid ($C_{75}H_{52}O_{46}$) was also used as a leaching agent.

These three preparates were dissolved into water so as to make a 0.05 % solution. According to Lutwick et al. [64] the concentration of leaf leachates obtained in a natural condition often reaches this value. All these solutions were made their pH value equal——pH = 5.5.

The tannic acid and polyuronic acid solutions were renewed in every 6 days, while the chestnut tannin solution was renewed in every 3 days, because the chestnut tannin solution was changeable in color.

7.3. Methods

i) Preparation of columns

Adsorbent materials were filled in columns, 3 cm in diameter and 50 cm in length, up to 35-40 cm in depth. The columns were dipped in a water tank and the adsorbents were moistened by capillary rise.

The following 5 divisions were set. Division 1-4 were set with an intention to elucidate influences of active alumina on mobilization of organic matter and active oxides. Division 5 was set for the purpose of comparison, because Kamigamo is different from Akaonji in its origin and relatively rich in active iron. In order to regulate the speed of percolation Kamigamo was mixed with the same part of Sand.

Division(Adsorbent)		Subdivision(Leaching agent)	Symbol
1. Akaonji	100 %	i. Chestnut tannin	1-i
		ii. Polyuronic acid	1-ii
		iii. Tannic acid	1-iii
2. Akaonji	26 %	i. Chestnut tannin	2-i
Sand	74 %	ii. Polyuronic acid	2-ii
		iii. Tannic acid	2-iii
3. Akaonji	10 %	i. Chestnut tannin	3-i
Sand	90 %	ii. Polyuronic acid	3-ii
		iii. Tannic acid	3-iii
4. Akaonji	3 %	i. Chestnut tannin	4-i
Sand	97 %	ii. Polyuronic acid	4-ii
		iii. Tannic acid	4-iii
5. Kamigamo	50 %	i. Chestnut tannin	5-i
Sand	50 %	ii. Polyuronic acid	5-ii
		iii. Tannic acid	5-iii

ii) Leaching practice

From 11th of July to 10th of August, 1960, 60 ml of each leaching solution was run through a column daily. The speed of percolation was regulated by a screw cock attached to the lower end of the column and it took about 5 or 6 hours to finish the percolation for all the columns.

The first percolate was discarded. From the 2nd to 29th every successive four percolates taken in order were brought together and

made up to 250 ml, and seven solutions (from the 1st to the 7th solution) thus obtained were analysed for iron and aluminum. The final 2 percolates and a 60 ml water leachate for washing were brought together and made up to 200 ml (the 8th solution), which was also analysed for iron and aluminum.

The 1st and 2nd solutions were both completely clear in every division, but in the 3rd solution (from the 10th to 13th percolate inclusively) a white precipitate appeared in the subdivision 4-ii. In the 4th and 5th solutions a similar precipitate appeared respectively in 5-ii and 3-ii. The precipitate in 5-ii was somewhat yellowish in color, and this might be due to contamination of clay leached out of the column.

Among the 5th solutions 4-i and 5-i showed a faint yellowish color which might be due to the organic matter percolated, and the color became deeper still in the solutions thenceforth. Among the 8th solutions a little bit of a white precipitate appeared in 2-ii.

iii) Method of analyses

In case of analysis of the leachate solutions an aliquot was taken and evaporated to dryness, then a small portion of 10 % $Mg(NO_3)_2$ solution was added and again brought to dryness. The evaporating dish was red-heated and after cooling the content was dissoved into a dilute HCl solution and made up to a certain volume. Iron and aluminum were analysed by the routine method.

After finishing the leaching the columns had been left standing until water content of them reached a certain level which enabled adsorbent materials easily to be taken out without disturbing columnar form. After being taken out the columnar adsorbent was divided into 6-8 separates by every 5 cm depth from the top and each separate was air-dried.

As to each separate, carbon, N/5-HCl soluble and concentrated-HCl (conc-HCl) soluble iron and aluminum were determined in duplicate samples.

N/5-HCl extraction was carried out as follows; 1g of a sample was weighed into a 100 ml Erlenmeyer flask and 50ml of a N/5-HCl solution was added. The flask was kept in a 40°C incubator for 5 hours with occasional shaking every 1 hour.

The procedure of conc-HCl extraction was different from the above

only in the following points: 1. The HCl solution was prepared by mixing 3 parts of the concentrated (about 12 N) HCl and 2 parts of water. 2. Flasks were kept in a 50°C incubator for 3 hours.

From the duplicate data L.S.D. was caulculated. The standard deviation was calculated after the control chart method.

7.4. Results and discussions

i) Results of the analyses of the leachate solutions

Analytical data for iron and aluminum oxides are given in Tables 7.1. and 7.2., respectively. As each datum was obtained from single determination, it is difficult to discuss the results in details. However, the total amount leached out indicates, at least, following facts.

1. The less the content of sesquioxides in a column is, the more easily they are leached out. In comparing Division 1 with 4 the R_2O_3 content of the former is 30 times as much as that of the latter, and yet the amount of R_2O_3 leached out of Division 4 is nearly equal to or rather higher than that of Division 1 in the corresponding subsivisions. This fact seems to coincide with the idea proposed by Kawaguchi and Matsuo [45] that "the quantitative ratio of the mobilizing organic agent to the material to be mobilized controls elluviation and illuviation". In the present case the quantity of the mobilizing agent is same for all the corresponding subdivisions and only the quantity of R_2O_3 is diddferent. Division 1, in which R_2O_3 is relatively predominating over the mobilizing agent, adsorbes the organic matter on the surface layer and the leachate contains relatively little R_2O_3. On the contrary in Division 4 the mobilizing agent seems to predominate over R_2O_3, and relatively much R_2O_3 in comparison with its original content is leached out. Moreover, in these cases active alumina is the main constituent of the active oxides and the author can reasonably extend the range of application of the principle proposed by Kawaguchi and Matsuo to Al_2O_3 beyond Fe_2O_3.

2. In Divisions 3, 4, and 5 polyuronic acid is more effective in leaching R_2O_3 than the other tannin preparations. This seems to be in some connection with appearance of the white precipitate in the leachate. The white precipitate is considered to be complex compounds of the polyuronic acids with R_2O_3, especially with Al_2O_3. Hcwever, the

extraordinary high figure for Division 5-ii is probably originated from clay contamination as mentioned above.

Table 7.1. The quantity of iron leached out of the columns (in γ Fe_2O_3)

Divisions	The leachate solutions 1	2	3	4	5	6	7	8	Total
1 - i	156	134	186	186	97	119	223	143	1244
1 - ii	312	283	186	238	127	89	127	202	1564
1 - iii	193	312	409	246	75	89	127	143	1591
2 - i	178	179	788	164	127	156	119	95	1806
2 - ii	156	164	737	82	112	149	89	196	1685
2 - iii	178	164	164	112	127	112	97	268	1172
3 - i	238	223	216	290	134	134	104	161	1500
3 - ii	201	208	536	283	253	238	156	178	2053
3 - iii	208	194	268	208	201	171	119	286	1655
4 - i	171	179	261	216	112	581	283	399	2202
4 - ii	201	283	201	305	283	402	365	333	2373
4 - iii	208	432	223	171	134	268	320	226	1982
5 - i	431	357	216	313	238	223	268	547	2593
5 - ii	609	357	2;6	350	1920	2225	3096	1808	10281
5 - iii	402	357	275	238	298	395	357	309	2631

Table 7.2. The quantity of aluminum leached out of the columns (in γ Al_2O_3)

Divisions	The leachate solutions 1	2	3	4	5	6	7	8	Total
1 - i	608	807	813	860	429	517	391	550	4975
1 - ii	571	817	827	833	575	462	660	623	5368
1 - iii	656	725	997	677	435	452	731	321	4994
2 - i	381	703	748	693	554	591	581	629	4880
2 - ii	467	775	687	215	435	585	329	538	4031
2 - iii	398	656	700	693	419	516	467	430	4279
3 - i	635	575	869	816	483	635	404	516	4933
3 - ii	705	555	944	1100	1420	1335	833	696	7588
3 - iii	602	646	848	1135	875	854	462	305	5727
4 - i	754	596	1110	986	446	419	329	343	4983
4 - ii	705	762	731	1660	1720	9160	1935	1700	18373
4 - iii	592	596	1320	970	698	629	661	326	5792
5 - i	710	684	800	764	725	425	516	1120	5744
5 - ii	1290	656	735	1670	6660	8320	10400	5330	35061
5 - iii	683	737	816	1375	527	650	752	538	6078

ii) Results of the analyses of the columns

The appearance of the columns after the end of the leaching practice was as follows:

In the subdivisions leached with chestnut tannin and tannic acid solutions, that is, i and iii, the columns showed dark reddish brown and dark gray color, respectively, at the upper part of the columns, where the organic matter was adsorbed, and the color became deeper with increment of the organic matter adsorption. However, there could not be noticed with the naked eyes any horizon differentiation caused by elluviation and illuviation of R_2O_3.

In the columns leached with polyuronic acid solution no sign was noticed which indicated any differentiation in either organic matter or R_2O_3 distribution.

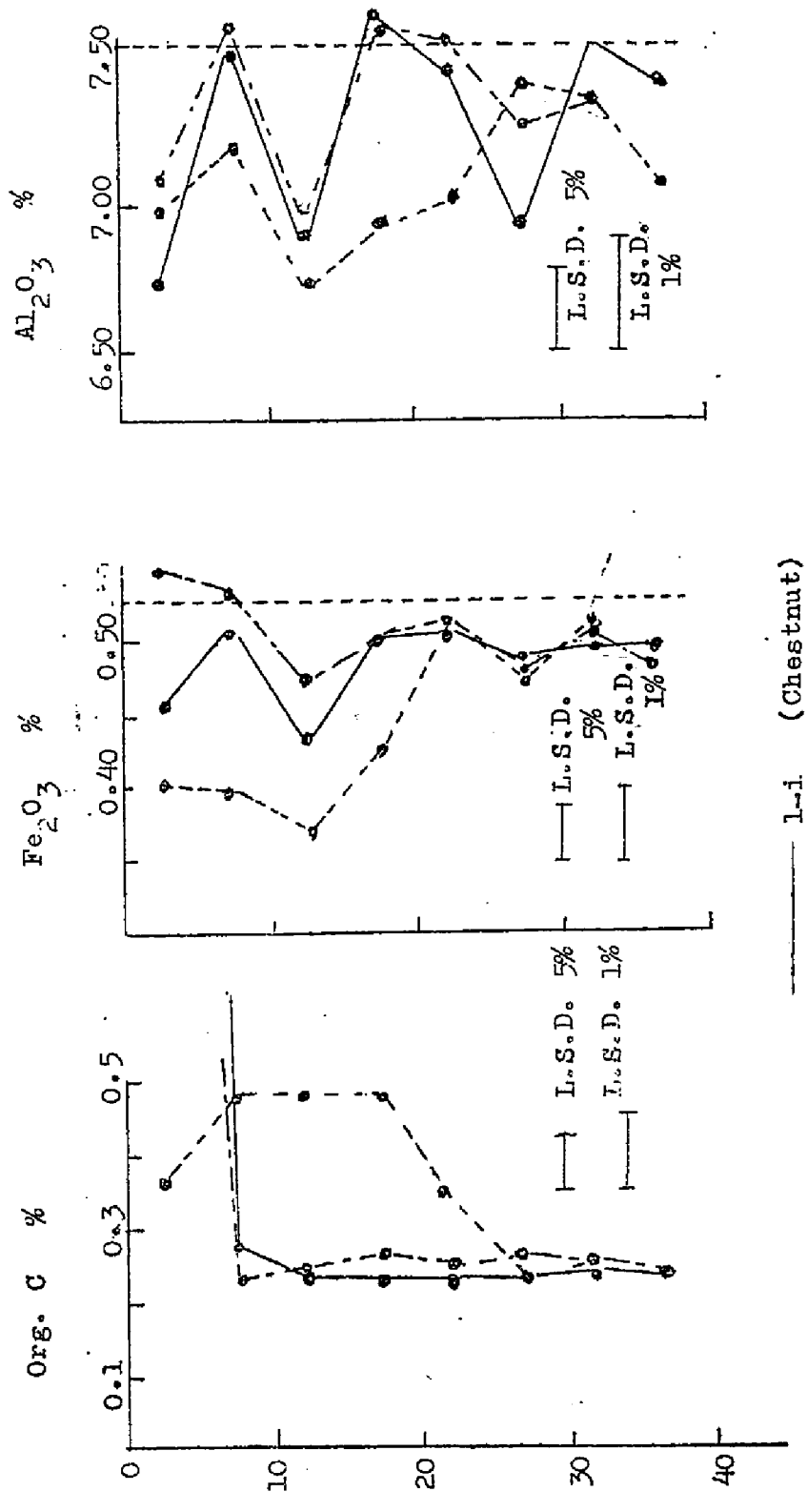
Hence all the columns were separated into 6-8 separates by every 5 cm length from the top of the column, and these separates were analyzed after air-drying.

The results of the analyses for organic carbon, N/5-HCl soluble Fe_2O_3 and Al_2O_3 are shown in Fig. 7.1. - 7.5., and for conc-HCl soluble sesquioxides in Fig. 7.6. - 7.10 in the order of the Division numbers.

With respect to the organic carbon (the data were recalculated so as to make the organic carbon content of the original column equal to zero), chestnut tannin and tannic acid distribute along the columns similarly in both Division 1 and 2. Adsorption or accumulation of those organic matter on the 1st and 2nd layers is conspicuous, while permeation into the lower part is very scarce. In Divisions 3 and 4 accumulation of chestnut tannin in the surface layers is less than that of tannic acid and decreasing tendency of the organic carbon towards the lower part is also less for chestnut than for tannic acid. In other words chestnut distributes relatively evenly along the column, while tannic acid content decreases abruptly from the upper to the lower part. This difference seems to be due to the difference in the organic matter composition of these materials, that is, the composition of chestnut is more complicated than that of tannic acid and, therefore, it involves various organic compounds which might have various tendency to be adsorbed on the column.

The mode of distribution of polyuronic acid is entirely different

Fig. 7.1. Distribution of adsorbed carbon and N/5-HCl soluble iron and aluminum oxide along the columns of Division 1



Note: In the graphs for sesquioxides, a broken line straightly drawn downwards indicates the original content of the oxide in the column.

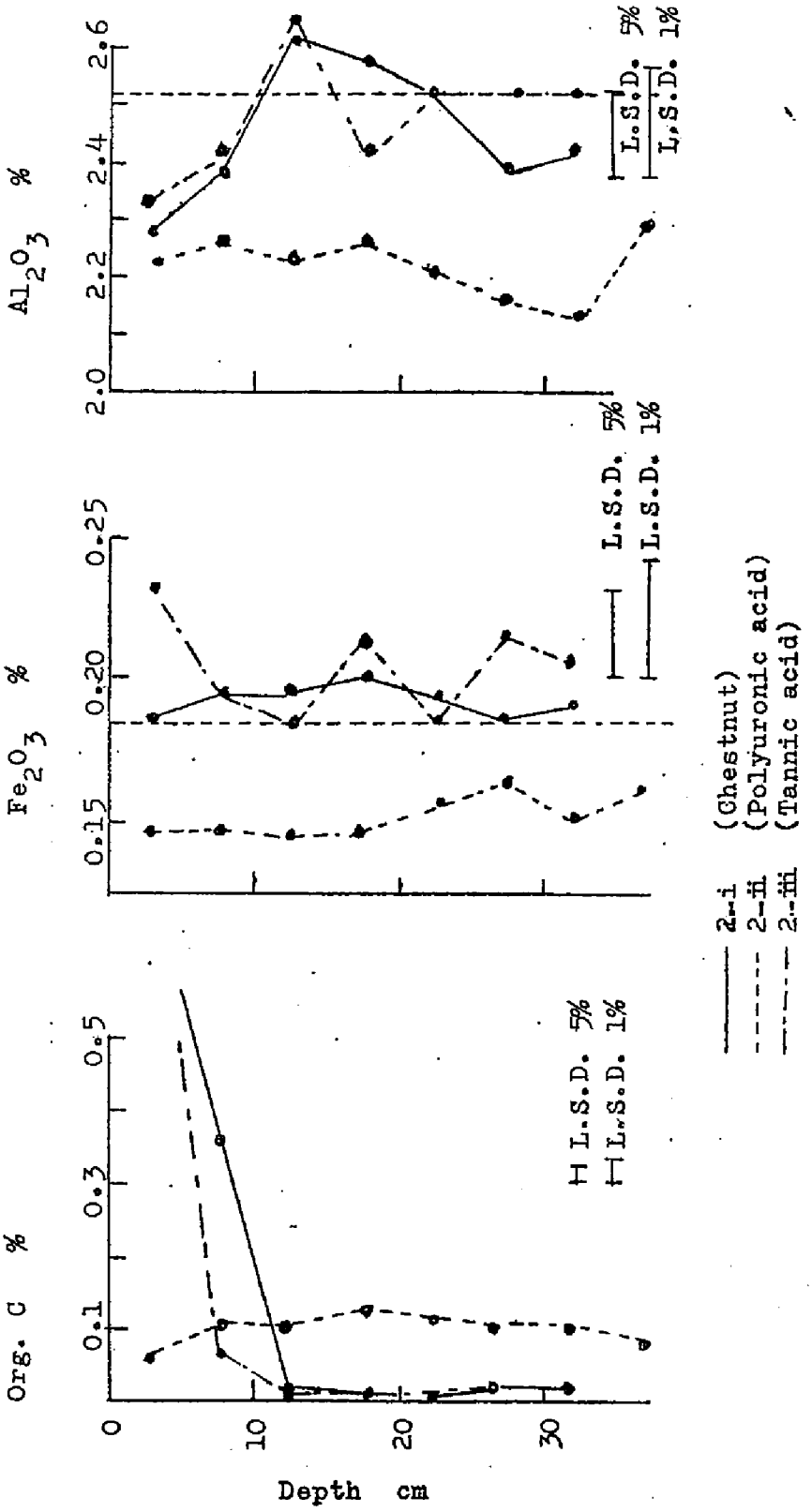


Fig. 7.2. Distribution of adsorbed carbon and N/5-HCl soluble iron and aluminum oxide along the columns of Division 2

Fig. 7.3. Distribution of adsorbed carbon and N/5-HCl soluble iron and aluminum oxide along the columns of Division 3

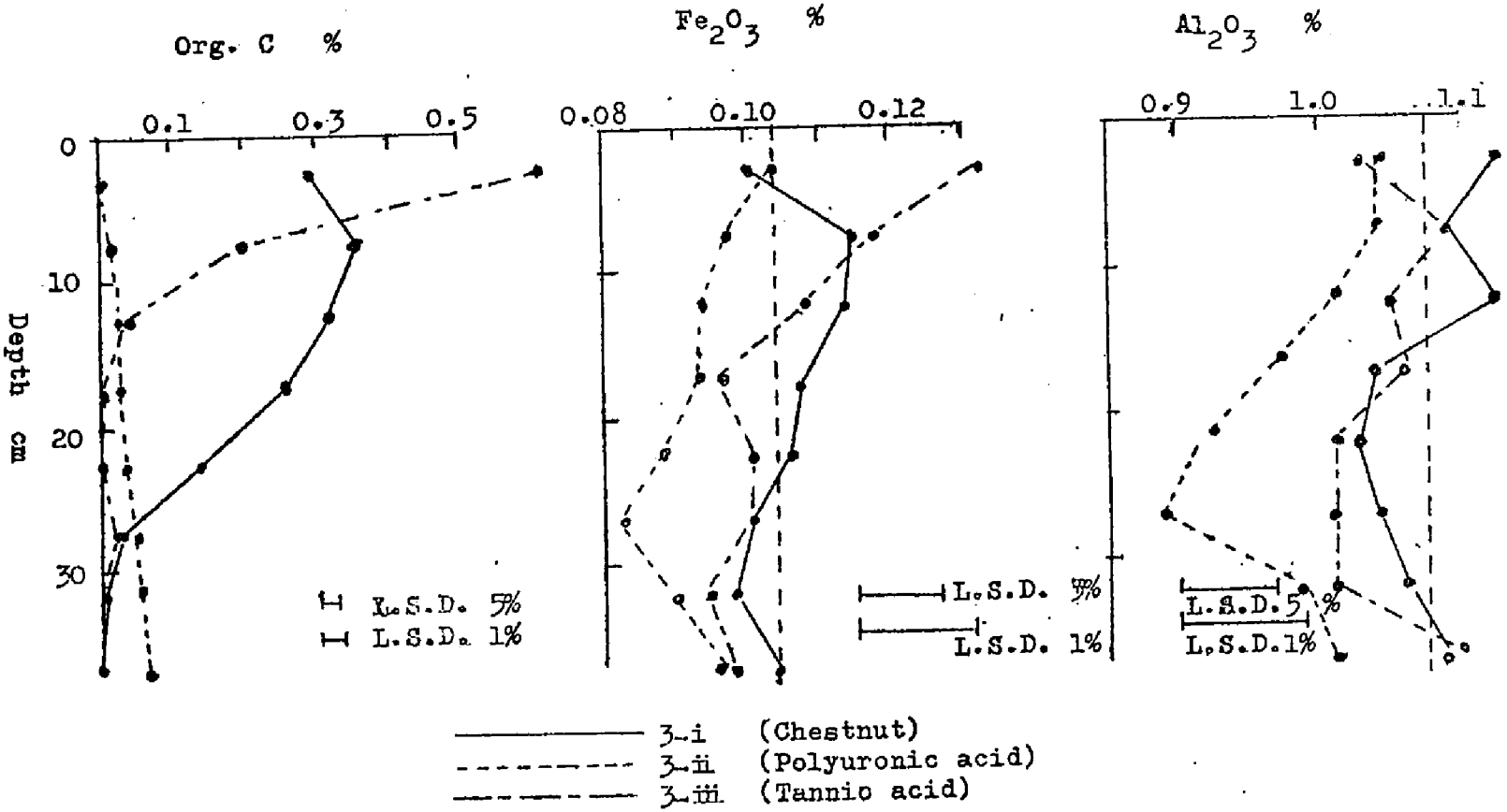


Fig. 7.4. Distribution of adsorbed carbon and N/5-HCl soluble iron and aluminum oxide along the columns of Division 4

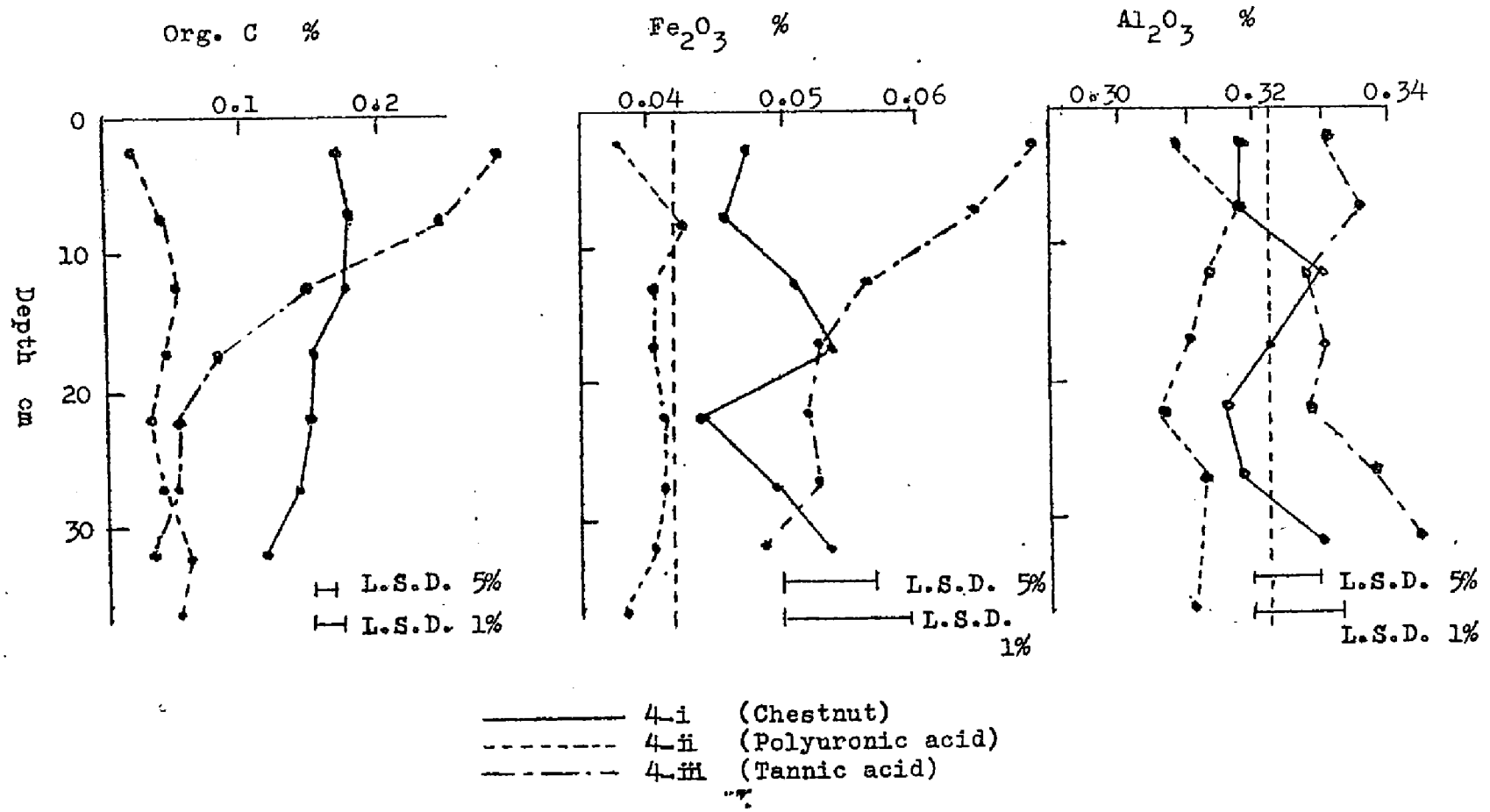
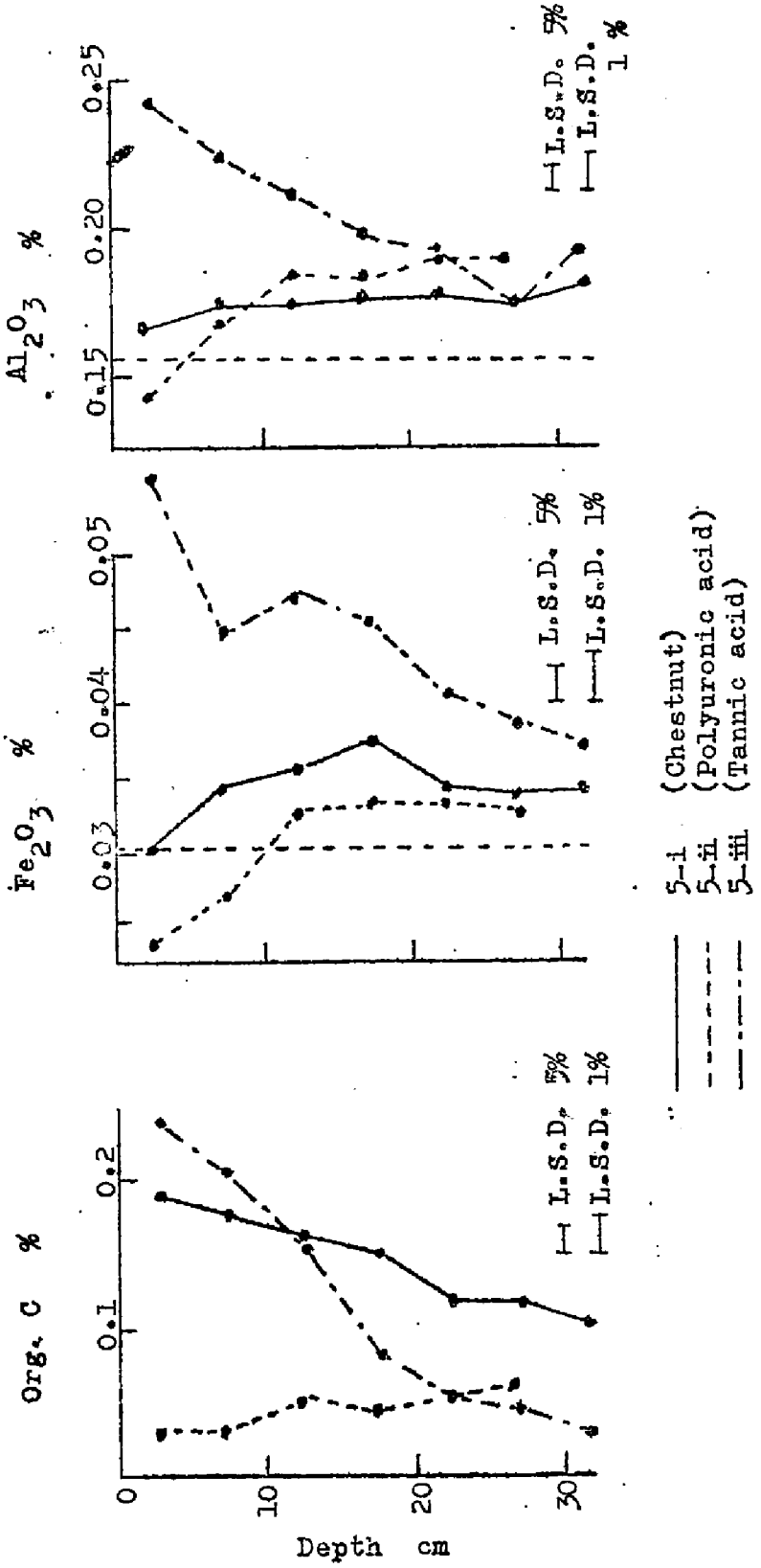


Fig. 7.5. Distribution of adsorbed carbon and N/5-HCl soluble iron and aluminum oxide along the columns of Division 5



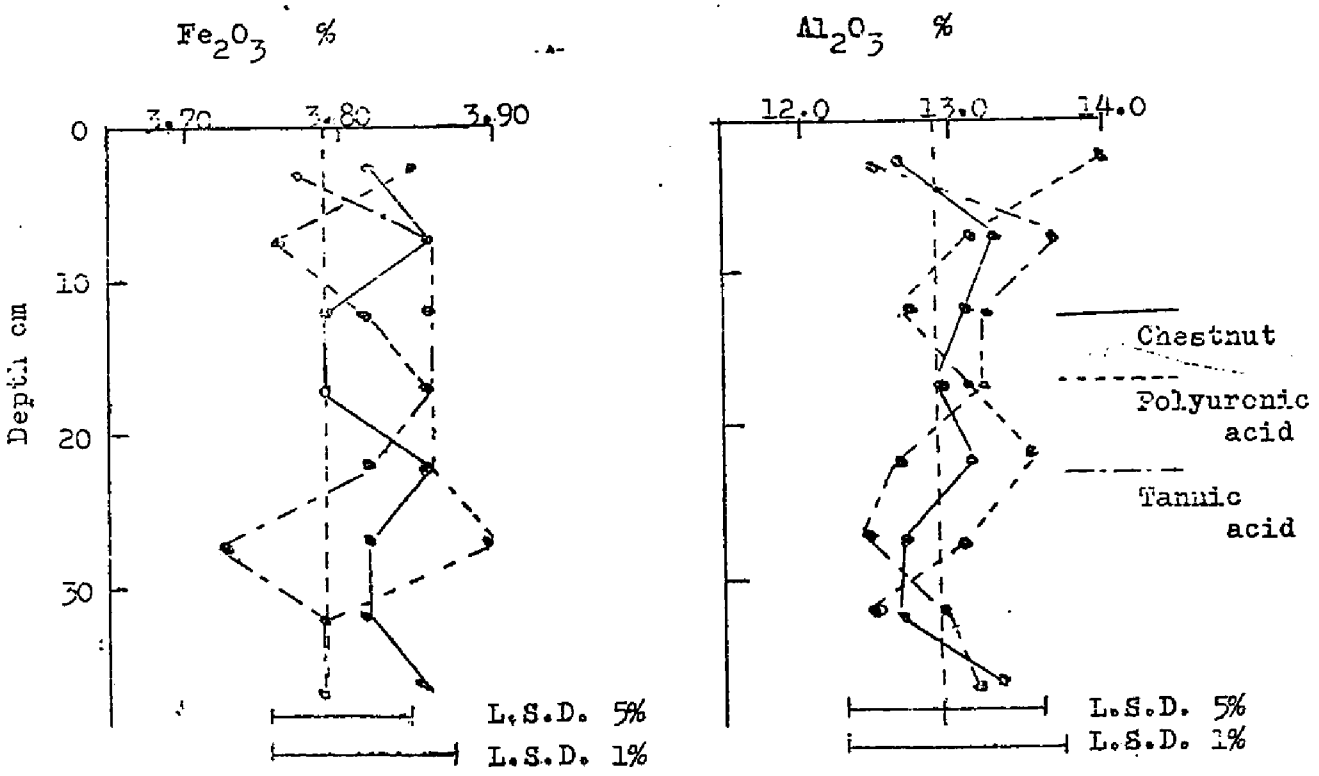


Fig. 7.6. Distribution of conc-HCl soluble iron and aluminum oxides along the columns of Division 1

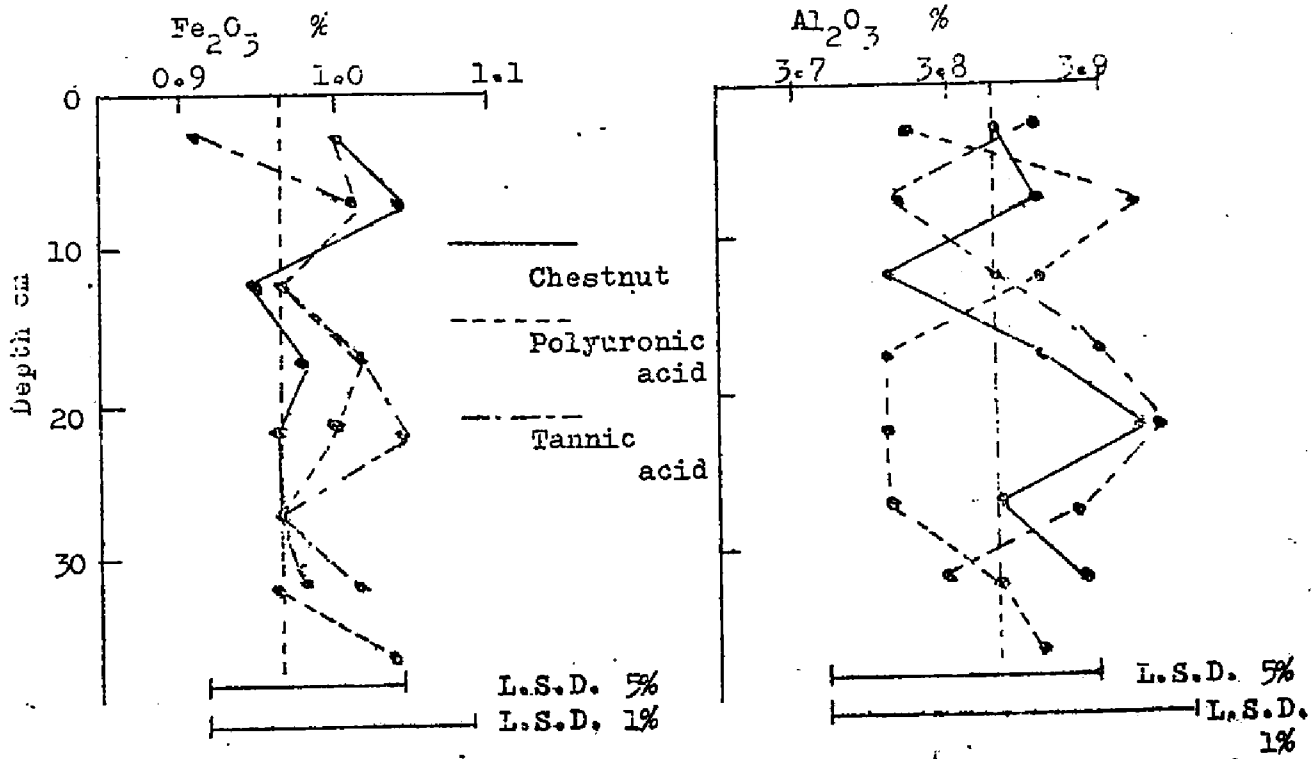


Fig. 7.7. Distribution of conc-HCl soluble iron and aluminum oxides along the columns of Division 2

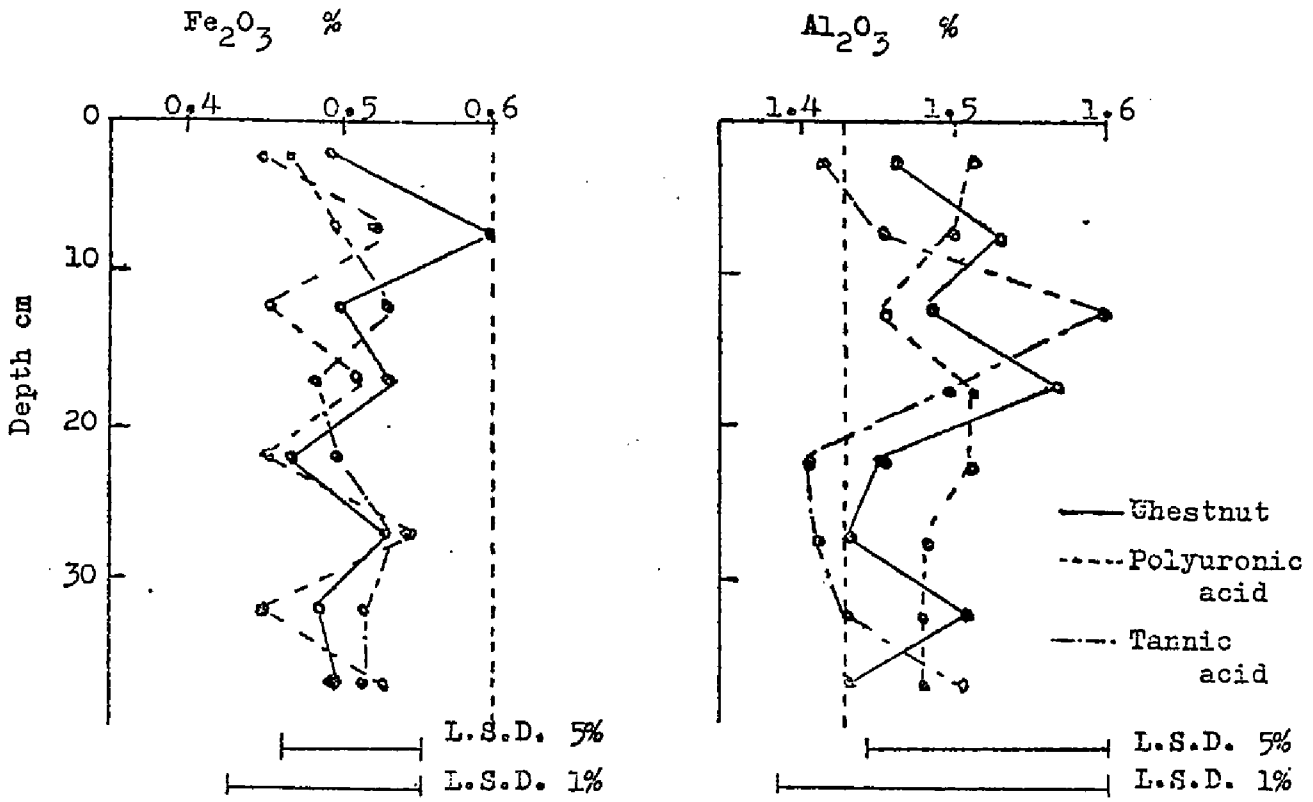


Fig. 7.8. Distribution of conc-HCl soluble iron and aluminum oxides along the columns of Division 3.

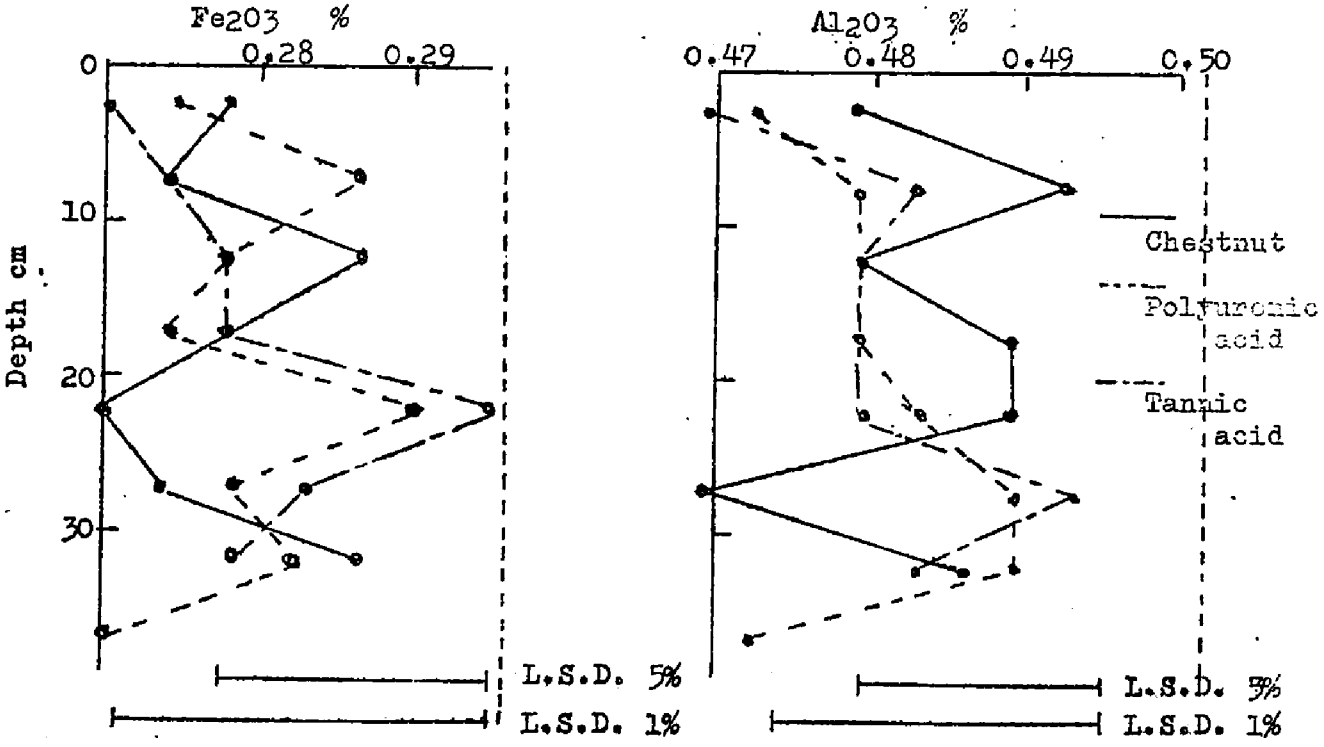


Fig. 7.9. Distribution of conc-HCl soluble iron and aluminum oxides along the columns of Division 4.

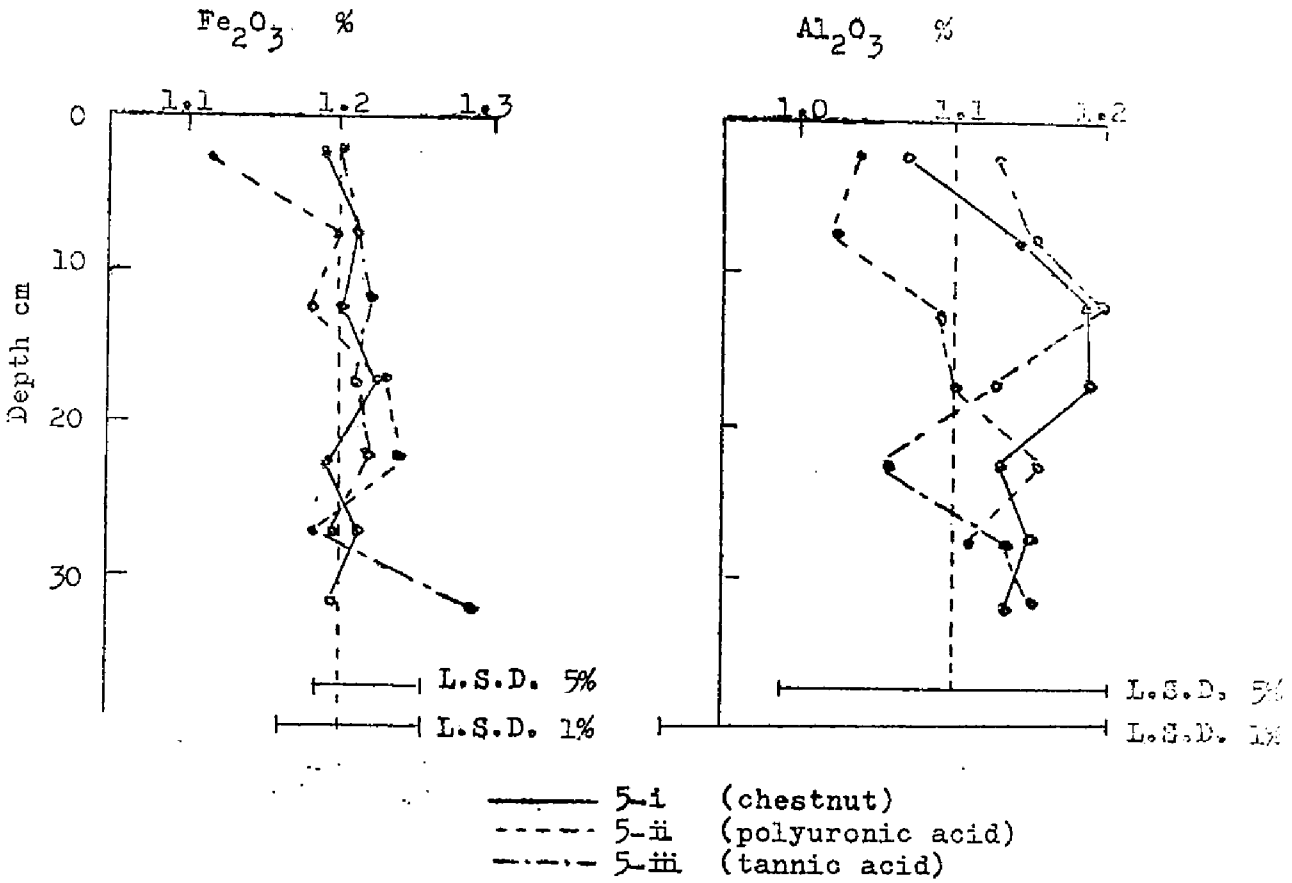


Fig. 7.10. Distribution of conc-HCl soluble iron and aluminum oxides along the columns of Division 5.

from the other agents. Even in Division 1 accumulation in the uppermost layer is less than that of the following 3 layers. In Divisions 2 - 5 there can not be noticed any clear difference in the organic carbon content throughout the column, or rather it increases gradually towards the lower part.

Generally, accumulation of polyuronic acid is always considerably less than that of the other. This might be due to various causes, for example, the molecular weight of the polyuronic acid used here. The molecular weight of the tannic acid is about 1700, and that of the chestnut tannin should be in the range from 1000 to 2000 as far as most of the chestnut tannin molecules can act as an effective tanning agent (according to Oshima, Y., Bul. Agr. Chem. Soc., Japan, Vol. 32 No.7, A81, 1958). In order to have a similar molecular weight the polyuronic acid should have 6 - 12 galacturonic acid units and this is likely to be the case in consideration of its solubility in cold water

and precipitability in a 70 % aqueous alcohol.

There might be a greater difference in the chemical behavior than in the molecular weight of these agents. Chestnut and tannic acid tend to precipitate more easily forming complex compounds with iron and aluminum, while polyuronic acid forms a more soluble complex, which migrates downwards and precipitates there partly. A part of the complex precipitates from leachate solutions as the white precipitate after being leached out.

Thus, as a matter of course, not only the quantitative ratio but also the quality of the mobilizing organic agents should be taken into account. In the natural soil formation the quality is determined mainly by the kind of vegetation, which is in turn determined mainly by the climatic conditions.

In Division 5 the mode of organic matter distribution is similar to that of Division 4 and hence one may say that the adsorption of organic carbon may be determined by the amount of easily soluble sesquioxides; for Mg-ribbon reducible active oxides and the conc-HCl soluble oxides are far more abundant in Division 5 than in Division 4.

Conc-HCl soluble sesquioxides were determined to know the overall changes in both active and inactive sesquioxides. The results showed that the conc-HCl soluble R_2O_3 content varied only insignificantly among the samples in a column, or even in a Division, that is, all the treatment did not cause any significant change in the distribution of sesquioxides soluble in the conc-HCl solution. However, with respect to Fe_2O_3 of Division 3 and both oxides of Division 4 (each oxide is contained originally less than 1 %), the content of the oxides after the leaching treatment tended to decrease slightly from the original one, but even in these cases sesquioxides distributed almost evenly along the columns.

The distribution of N/5-HCl soluble sesquioxides has following features:

1. Columns leached with polyuronic acid solution have somewhat lower content of N/5-HCl soluble R_2O_3. This is in some measure correspondent with the fact that the leachates, especially of Subdivisions 3-ii, 4-ii. and 5-ii, contained relatively much sesquioxides.
2. N/5-HCl soluble Fe_2O_3 apparently tends to accumulate in surface layers, especially in Subdivisions leached with tannic materials.

The tendency is more marked in Divisions 3,4 and 5 which are relatively poor in the easily soluble iron. It is easily infered that the tannic materials reduces otherwise difficultly soluble iron and this increases mobile iron like N/5-HCl soluble one. This inference is supported by the following observation that the acid extracts obtained from layers containing much tannic materials have much ferrous iron which is able to react directly with orthophenanthroline in the analytical procedure adopted by the author. This fact suggests a great contribution of polyphenols to mobilization of iron through their reducing ability. Recently the importance was recognized also by Coulson et al. [25] in their experiments. They thought much of reducing power of catechins in mobilizing iron.

It is also noticed, particularly in Divisions 4 and 5, that mobility of aluminum is increased considerably through the process of reduction and mobilization of iron. This is considered to be due to removal of iron oxides which have been preventing dissolution of the alumina.

In several columns significant difference was noticed in the contents of N/5-HCl soluble R_2O_3 among the layers, but as mentioned in the case of conc-HCl soluble R_2O_3 essential elluviation and illuviation had not occured yet in the present study.

Rather it is noticeable that the reducing power of the tannic materials caused elevation of mobility of iron directly and that of aluminum indirectly, and this must be the prerequisite for the essential elluviation and illuviation to follow after.

Coulson et al.[25] limited the effectiveness of catechins in mobilization only to iron oxides, but in the present experiment not only polyuronic acid generally had high mobilizing ability for aluminum, but the tannic materials were also considerably effective in mobilizing aluminum. This might be due to non-uniform composition of the tannin preparates used and there should be involved some compounds which are able to form complex with aluminum.

Microbial activity on the columns during the term of the experiment seemed to be negligible, and so it was not refered to.

7.5. Summary

Plant materials having chelating ability like polyphenols and

polyuronides were examined relative to their effectiveness in soil formation, especially in mobilization of sesquioxides. Solutions of a chestnut tanrin preparate, a commercial tannic acid and polyuronic acid preparate obtained from citrus pectin were passed through columns containing various proportion of a glassy volcanic ash material (Akaonji) and a polishing sand. In one division a weathered material of a Paleozoic sandstone was used instead of Akaonji. The results are summarized as follows.

1. From the analytical data of the leachates it was shown that the higher the original R_2O_3 content was, the less the amount appeared in the leachate, and thus "the concept of the quantitative ratio" proposed by Kawaguchi and Matsuo was supported.

2. Both tannic materials and polyuronic acid were more or less effective in mobilizing both iron and aluminum. Polyuronic acid was the most effective leaching agent among them and seemed to form complex compounds with aluminum.

3. Tannic materials seemed to form complexes low in mobility and were kept, for the most part, in the column. Moreover, they reduced iron oxides and the easily soluble iron was apparently accumulated in the surface layers, though the essential elluviation and illuviation process was not detectable from the analytical data of conc-HCl soluble R_2O_3. However, the mobile iron produced by reduction might be the prerequisite of the essential horizon differentiation. A part of aluminum also obtained mobility as a result of the removal of the iron oxides.

4. Polyuronic acid complexes were relatively high in mobility and permeated into the lower part of the column. Easily soluble iron and aluminum were less in the polyuronic acid columns than in the tannic material columns. However, in conc-HCl soluble R_2O_3 content there could not be found any significant difference between the columns leached respectively with uronic and tannic materials.

Chapter 8

SUMMARY AND CONCLUSION

In the present thesis the following two subjects were investigated :

1) Association of soil humus with soil mineral constituents, especially with sesquioxides. In the course of discussion the problem of the structure of soil humic substances was also dealt with.

2) The significance of polyphenols both as a soil former and as a constituent of humus. In connection with the second-mentioned role of polyphenols, efforts were made to elucidate the peculiarity involved in the humus-forming process in humus volcanic ash soils.

The most important results obtained from these studies are the followings:

1) In the humus composition of the main soil groups in Japan, the fraction loosely bound to sesquioxides is predominating and this fact may be one of the most essential features of the soils in Japan formed under fairly humid conditions.

2) One of the bonding mechanisms between soil humus and sesquioxides is complex-formation (probably chelation). In the complex-formation humic acids have a low complexing ability and are ready to precipitate, while fulvic acids show a high ability to form water soluble complexes with iron and aluminum, and thus seem to have a greater effectiveness in the podzol forming process.

3) It was confirmed that cation exchange capacity of humic acids increases along with the progress of the humification. The author suggested that this fact is not concordant with the concept of humus structure, effective as it is in many respects, proposed by the Russian school.

4) Allophanic volcanic ash materials like Akaonji exert an influence on the oxidative polymerization of polyphenols as an adsorption-catalyst. It was suggested that the same influence might be exerted by the parent materials on the humus-forming processes in the humus volcanic ash soil, and that this might be one of the most essential peculiarities involved in the soil.

5) The concept of "quantitative ratio" proposed by Kawaguchi and

Matsuo in connection with mobilization and immobilization of iron, was found to be adaptable also to aluminum oxides.

6) It was suggested that the effectiveness of polyphenols in soil-forming process consists, first of all, in their reducing power.

Studies on the soil organic matter from the standpoint of soil genesis must be further developed, because the organic matter has the greatest influence on the genesis and only the genetical understanding of a soil can afford a mean to improve fertility of the soil.

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